Devi, S.
09/830026

-key terms

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     FILE 'REGISTRY' ENTERED AT 11:35:49 ON 10 JUL 2003
                  E INVASIN/CN 5
L1             38 S INVASIN ?/CN
                  E GUANIDINE HYDROCHLORIDE/CN 5
L2              1 S E3
                  E UREA/CN 5
L3              1 S E3
L4              2 S L2 OR L3

     FILE 'HCAPLUS' ENTERED AT 11:40:14 ON 10 JUL 2003
L1             38 SEA FILE=REGISTRY ABB=ON  PLU=ON  INVASIN ?/CN
L2              1 SEA FILE=REGISTRY ABB=ON  PLU=ON  "GUANIDINE HYDROCHLORID
                  E"/CN
L3              1 SEA FILE=REGISTRY ABB=ON  PLU=ON  UREA/CN
L4              2 SEA FILE=REGISTRY ABB=ON  PLU=ON  L2 OR L3
L5            313 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR INVASIN OR
                  INV(S)INVASIN
L6              6 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5 AND (L4 OR DENATUR?
                  OR GUANIDIN?(W)(HYDROCHLORIDE OR HYDRO CHLORIDE OR HCL)
                  OR UREA OR DETERGENT)


L1             38 SEA FILE=REGISTRY ABB=ON  PLU=ON  INVASIN ?/CN
L5            313 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR INVASIN OR
                  INV(S)INVASIN
L7             16 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5(S)(PURE OR PURIF?)


L8             21 L6 OR L7

L8   ANSWER 1 OF 21  HCAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2002:815744  HCAPLUS
DOCUMENT NUMBER:          138:52543
TITLE:                    Intracellular growth of Legionella pneumophila
                          gives rise to a differentiated form dissimilar
                          to stationary-phase forms
AUTHOR(S):                Garduno, Rafael A.; Garduno, Elizabeth; Hiltz,
                          Margot; Hoffman, Paul S.
CORPORATE SOURCE:         Department of Microbiology and Immunology and
                          Division of Infectious Diseases, Department of
                          Medicine, Faculty of Medicine, Dalhousie
                          University, Halifax, NS, B3H-4H7, Can.
SOURCE:                   Infection and Immunity (2002), 70(11), 6273-6283
                          CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER:                American Society for Microbiology
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   When Legionella pneumophila grows in HeLa cells, it alternates
     between a replicative form and a morphol. distinct "cyst-like" form
     termed MIF (mature intracellular form).  MIFs are also formed in
     natural amoebic hosts and to a lesser extent in macrophages, but
     they do not develop in vitro.  Since MIFs accumulate at the end of
     each growth cycle, we investigated the possibility that they are in
     vivo equiv. of stationary-phase (SP) bacteria, which are enriched
     for virulence traits.  By electron microscopy, MIFs appeared as
     short, stubby rods with an electron-dense, laminar outer membrane
     layer and a cytoplasm largely occupied by inclusions of
     poly-.beta.-hydroxybutyrate and laminations of internal membranes
```

originating from the cytoplasmic membrane. These features may be responsible for the bright red appearance of MIFs by light microscopy following staining with the phenolic Gimenez stain. In contrast, SP bacteria appeared as dull red rods after Gimenez staining and displayed a typical gram-neg. cell wall ultrastructure. Outer membranes from MIFs and SP bacteria were equiv. in terms of the content of the peptidoglycan-bound and disulfide bond cross-linked OmpS porin, although addnl. proteins, including Hsp60 (which acts as an **invasin** for HeLa cells), were detected only in prepns. from MIFs. Proteomic anal. revealed differences between MIFs and SP forms; in particular, MIFs were enriched for an .apprx.20-kDa protein, a potential marker of development. Compared with SP bacteria, MIFs were 10-fold more infectious by plaque assay, displayed increased resistance to rifampin (3- to 5-fold) and gentamicin (10- to 1,000-fold), resisted **detergent** -mediated lysis, and tolerated high pH. Finally, MIFs had a very low respiration rate, consistent with a decreased metabolic activity. Collectively, these results suggest that intracellular L. pneumophila differentiates into a cyst-like, environmentally resilient, highly infectious, post-SP form that is distinct from in vitro SP bacteria. Therefore, MIFs may represent the transmissible environmental forms assocd. with Legionnaires' disease.

REFERENCE COUNT: 68 THERE ARE 68 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:855411 HCAPLUS
DOCUMENT NUMBER: 136:196501
TITLE: Cloning, expression, and **purification** of the uropathogenic Escherichia coli **invasin** DraD
AUTHOR(S): Zalewska, Beata; Piatek, Rafal; Cieslinski, Hubert; Nowicki, Bogdan; Kur, Jozef
CORPORATE SOURCE: Department of Microbiology, Technical University of Gdansk, Gdansk, 80-952, Pol.
SOURCE: Protein Expression and Purification (2001), 23(3), 476-482
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In this study we presented a very efficient expression system, based on pET30LIC/Ek vector, for producing DraD **invasin** of the uropathogenic Escherichia coli and a one-step chromatog. **purifn.** procedure for obtaining **pure** recombinant protein (DraD-C-His6). This protein has a mol. wt. of 14,818 and calcd. pI of 6.6. It contains a polyhistidine tag at the C-terminus (13 addnl. amino acids) that allowed single-step isolation by Ni affinity chromatog. Also, we obtained specific antibodies against DraD invasin to develop tools for characterizing the expression and biol. function of this protein. The amt. and quality of DraD-C-His6 fusion protein purified from E. coli overexpression system seems to be fully appropriate for crystallog. studies (sol. form), and for establishing role of the protein in bacterium (cultured cell line interaction and in the internalization process) and for obtaining rabbit polyclonal antisera (insol. form). (c) 2001 Academic Press.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE

FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:711693 HCAPLUS
DOCUMENT NUMBER: 136:2833
TITLE: Actin-based motility is sufficient for bacterial membrane protrusion formation and host cell uptake
AUTHOR(S): Monack, Denise M.; Theriot, Julie A.
CORPORATE SOURCE: Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, 94305-5307, USA
SOURCE: Cellular Microbiology (2001), 3(9), 633-647
CODEN: CEMIF5; ISSN: 1462-5814
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Shigella flexneri replicates in the cytoplasm of host cells, where it nucleates host cell actin filaments at one pole of the bacterial cell to form a "comet tail" that propels the bacterium through the host's cytoplasm. To det. whether the ability to move by actin-based motility is sufficient for subsequent formation of membrane-bound protrusions and intercellular spread, we conferred the ability to nucleate actin on a heterologous bacterium, Escherichia coli. Previous work has shown that IcsA (VirG), the mol. that is necessary and sufficient for actin nucleation and actin-based motility, is distributed in a unipolar fashion on the surface of S. flexneri. Maintenance of the unipolar distribution of IcsA depends on both the S. flexneri outer membrane protease IcsP (SopA) and the structure of the lipopolysaccharide (LPS) in the outer membrane. We co-expressed IcsA and IcsP in two strains of E. coli that differed in their LPS structures. The E. coli were engineered to invade host cells by expression of **invasin** from Yersinia pseudotuberculosis and to escape the phagosome by incubation in **purified** listeriolysin O (LLO) from Listeria monocytogenes. All E. coli strains expressing IcsA replicated in host cell cytoplasm and moved by actin-based motility. Actin-based motility alone was sufficient for the formation of membrane protrusions and uptake by recipient host cells. The presence of IcsP and an elaborate LPS structure combined to enhance the ability of E. coli to form protrusions at the same frequency as S. flexneri, quant. reconstituting this step in pathogen intercellular spread in a heterologous organism. The frequency of membrane protrusion formation across all strains tested correlates with the efficiency of unidirectional actin-based movement, but not with bacterial speed.
REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:535219 HCAPLUS
DOCUMENT NUMBER: 135:268939
TITLE: Expression, refolding and crystallization of the OpcA **invasin** from Neisseria meningitidis
AUTHOR(S): Prince, S. M.; Feron, C.; Janssens, D.; Lobet,

Y.; Achtman, M.; Kusecek, B.; Bullough, P. A.; Derrick, J. P.
CORPORATE SOURCE: Department of Biomolecular Sciences, UMIST, Manchester, UK
SOURCE: Acta Crystallographica, Section D: Biological Crystallography (2001), D57(8), 1164-1166
CODEN: ABCRE6; ISSN: 0907-4449
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB OpcA is an integral outer membrane from the Gram-neg. pathogen Neisseria meningitidis that plays a role in adhesion of meningococci to host cells. The protein was overexpressed in Escherichia coli in an insol. form and a procedure developed for refolding by rapid diln. from **denaturant** into **detergent** soln. The refolded material was identical to native OpcA isolated from meningococci, as judged by overall mol. wt., migration on SDS-PAGE and reaction against monoclonal antibodies. Both native and recombinant OpcA crystd. under similar conditions to give an orthorhombic crystal form (P21212), with unit-cell parameters a = 96.9, b = 46.3, c = 74.0 .ANG.. Complete data sets of reflections were collected from native and refolded OpcA to 2.0 .ANG. resoln.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:374740 HCAPLUS
DOCUMENT NUMBER: 135:151269
TITLE: Isolation and characterization of a Shigella flexneri invasin complex subunit vaccine
AUTHOR(S): Turbyfill, K. Ross; Hartman, Antoinette B.; Oaks, Edwin V.
CORPORATE SOURCE: Department of Enteric Infections, Walter Reed Army Institute of Research, Silver Spring, MD, 20910-7500, USA
SOURCE: Infection and Immunity (2000), 68(12), 6624-6632
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The invasiveness and virulence of Shigella spp. are largely due to the expression of plasmid-encoded virulence factors, among which are the invasion plasmid antigens (Ipa proteins). After infection, the host immune response is directed primarily against lipopolysaccharide (LPS) and the virulence proteins (IpaB, IpaC, and IpaD). Recent observations have indicated that the Ipa proteins (IpaB, IpaC, and possibly IpaD) form a multiprotein complex capable of inducing the phagocytic event which internalizes the bacterium. We have isolated a complex of invasins and LPS from water-extractable antigens of virulent shigellae by ion-exchange chromatog. Western blot anal. of the complex indicates that all of the major virulence antigens of Shigella, including IpaB, IpaC, and IpaD, and LPS are components of this macromol. complex. Mice or guinea pigs immunized intranasally with **purified invasin** complex (invaplex), without any addnl. adjuvant, mounted a significant IgG and IgA antibody response against the Shigella virulence antigens and LPS. The virulence-specific

response was very similar to that previously noted in primates infected with shigellae. Guinea pigs (keratoconjunctivitis model) or mice (lethal lung model) immunized intranasally on days 0, 14, and 28 and challenged 3 wk later with virulent shigellae were protected from disease.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 6 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:277999 HCAPLUS
DOCUMENT NUMBER: 132:307246
TITLE: Method for the production of **purified invasin** protein and use thereof
INVENTOR(S): Picking, William D.; Picking, Wendy D.; Oaks, Edwin V.
PATENT ASSIGNEE(S): St. Louis University, USA
SOURCE: PCT Int. Appl., 78 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000023462	A1	20000427	WO 1999-US24931	19991021
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1131338	A1	20010912	EP 1999-970664	19991021
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.:
US 1998-105085P P 19981021
US 1999-136754P P 19990601
WO 1999-US24931 W 19991021

AB A method for prodn. of highly **purified invasin** proteins is disclosed. The **invasin** proteins are recombinant IpaC or SipC derived from Shigella spp., Salmonella spp., and enteroinvasive Escherichia coli. Also disclosed are vaccine and adjuvant compns. comprising highly **purified invasin** proteins and the use of highly **purified** adjuvant proteins to induce an immune response and for delivery of therapeutic and diagnostic agents or drugs.

IT **169183-65-7**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; recombinant **invasin** protein for use as adjuvant and for delivery of vaccine, therapeutic and diagnostic agents)

IT **50-01-1, Guanidine hydrochloride**
57-13-6, Urea, biological studies

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(protein **denaturant**; recombinant **invasin** protein for use as adjuvant and for delivery of vaccine, therapeutic and diagnostic agents)
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:227460 HCAPLUS
DOCUMENT NUMBER: 132:264091
TITLE: Use of purified Invaplex from gram negative bacteria as a vaccine
INVENTOR(S): Oaks, Edwin V.; Turbyfill, Kevin Ross; Hartman, Antoinette Berrong
PATENT ASSIGNEE(S): Walter Reed Army Institute of Research, USA
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000018355	A2	20000406	WO 1999-US22772	19990928
WO 2000018355	A3	20001123		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000011999	A1	20000417	AU 2000-11999	19990928
EP 1198245	A2	20020424	EP 1999-969665	19990928
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
US 6245892	B1	20010612	US 1999-408011	19990929
US 6277379	B1	20010821	US 1999-407330	19990929
US 2001009957	A1	20010726	US 2001-772878	20010131

PRIORITY APPLN. INFO.:
US 1998-102397P P 19980930
US 1998-102398P P 19980930
US 1999-136190P P 19990527
WO 1999-US22772 W 19990928
US 1999-408011 A3 19990929

AB A novel compn. comprising Invaplex from gram-neg. bacteria is described and is effective as a vaccine against gram-neg. bacterial infection.

L8 ANSWER 8 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:197695 HCAPLUS
DOCUMENT NUMBER: 131:16332
TITLE: A region of the Yersinia pseudotuberculosis invasin protein enhances integrin-mediated

uptake into mammalian cells and promotes self-association
AUTHOR(S): Dersch, Petra; Isberg, Ralph R.
CORPORATE SOURCE: Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA, 02111, USA
SOURCE: EMBO Journal (1999), 18(5), 1199-1213
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Invasin allows efficient entry into mammalian cells by Yersinia pseudotuberculosis. The C-terminal 192 amino acids of invasin are essential for binding of .beta.1 integrin receptors and subsequent uptake. By analyzing the internalization of latex beads coated with invasin derivs., an addnl. domain of invasin was shown to be required for efficient bacterial internalization. A monomeric deriv. encompassing the C-terminal 197 amino acids was inefficient at promoting entry of latex beads, whereas dimerization of this deriv. by antibody significantly increased uptake. By using the DNA-binding domain of .lambda. repressor as a reporter for invasin self-interaction, a region of the invasin protein located N-terminal to the cell adhesion domain of invasin was demonstrated to self-assoc. Chem. crosslinking studies of **purified** and surface-exposed **invasin** proteins and the dominant-interfering effect of a non-functional **invasin** deriv. are consistent with the presence of a self-assocn. domain that is located within the region of **invasin** that enhances bacterial uptake. Thus, interaction of homomultimeric invasin with multiple integrins establishes tight adherence and receptor clustering, thus providing a signal for internalization.
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 9 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:488569 HCAPLUS
DOCUMENT NUMBER: 129:199465
TITLE: Recombinant Soluble Human .alpha.3.beta.1 Integrin: **Purification**, Processing, Regulation, and Specific Binding to Laminin-5 and **Invasin** in a Mutually Exclusive Manner
AUTHOR(S): Eble, Johannes A.; Wucherpfennig, Kai W.; Gauthier, Laurent; Dersch, Petra; Krukonis, Eric; Isberg, Ralph R.; Hemler, M. E.
CORPORATE SOURCE: Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, 02115, USA
SOURCE: Biochemistry (1998), 37(31), 10945-10955
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Using insect cells, we expressed large quantities of sol. human integrin .alpha.3.beta.1 ectodomain heterodimers, in which cytoplasmic and transmembrane domains were replaced by Fos and Jun dimerization motifs. In direct ligand binding assays, sol. .alpha.3.beta.1 specifically bound to laminin-5 and laminin-10, but

not to laminin-1, laminin-2, fibronectin, various collagens, nidogen, thrombospondin, or complement factors C3 and C3b. Sol. .alpha.3.beta.1 integrin also bound to invasin, a bacterial surface protein, that mediates entry of Yersinia species into the eukaryotic host cell. Invasin completely displaced laminin-5 from the .alpha.3.beta.1 integrin, suggesting sterically overlapping or identical binding sites. In the presence of 2 mM Mg2+, .alpha.3.beta.1's binding affinity for invasin (Kd = 3.1 nM) was substantially greater than its affinity for laminin-5 (Kd > 600 nM). Upon addn. of 1 mM Mn2+, or activating antibody 9EG7, binding affinity for both laminin-5 and invasin increased by about 10-fold, whereas the affinity decreased upon addn. of 2 mM Ca2+. Thus, functional regulation of the purified sol. integrin .alpha.3.beta.1 ectodomain heterodimer resembles that of wild-type membrane-anchored .beta.1 integrins. The integrin .alpha.3 subunit was entirely cleaved into disulfide-linked heavy and light chains, at a newly defined cleavage site located C-terminal of a tetrabasic RRRR motif. Within the .alpha.3 light chain, all potential N-glycosylation sites bear N-linked mannose-rich carbohydrate chains, suggesting an important structural role of these sugar residues in the stalk-like region of the integrin heterodimer. In conclusion, studies of our recombinant .alpha.3.beta.1 integrin have provided new insights into .alpha.3.beta.1 structure, ligand binding function, specificity, and regulation.

REFERENCE COUNT: 70 THERE ARE 70 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 10 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:584512 HCAPLUS
DOCUMENT NUMBER: 125:270180
TITLE: A region of the Yersinia pseudotuberculosis invasin protein that contributes to high affinity binding to integrin receptors
AUTHOR(S): Saltman, Laura H.; Lu, Yin; Zaharias, Evanthia M.; Isberg, Ralph R.
CORPORATE SOURCE: Howard Hughes Med. Inst., Tufts Univ. Sch. Med., Boston, MA, 02111, USA
SOURCE: Journal of Biological Chemistry (1996), 271(38), 23438-23444
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The entry of Yersinia pseudotuberculosis into cultured mammalian cells is mediated by the bacterial protein invasin. The mammalian receptors for invasin are five .beta.1-chain integrins. Site-directed mutagenesis of the aspartate and lysine residues in the 192-amino acid integrin-binding domain of invasin was performed to identify regions, in addn. to the previously characterized 903-913 region, that are important for integrin binding. One mutation, D811A, resulted in depressed ability of **invasin** to bind **purified** .alpha.5.beta.1 and to promote bacterial entry. Further mutational anal. of Asp-811 indicated that an oxygen-contg. side chain is required at this position. A second nearby residue, Phe-808, was also shown to be important for integrin binding, as an alanine substitution at this site had properties

similar to the Asp-811 mutation. This mutational anal. has therefore identified a second region that, in conjunction with residues 903-913, is required for wild-type levels of integrin binding. The contribution to binding by two noncontiguous sites in the primary sequence parallels results that indicate two domains of fibronectin are involved in integrin binding.

L8 ANSWER 11 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:577834 HCAPLUS
DOCUMENT NUMBER: 125:214677
TITLE: Rapid detection of virulence-associated factors
INVENTOR(S): Thorne, Grace M.
PATENT ASSIGNEE(S): Children's Medical Center Corporation, USA
SOURCE: U.S., 9 pp., Cont. of U.S. Ser. No. 963,724, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5552294	A	19960903	US 1994-279832	19940725
PRIORITY APPLN. INFO.:			US 1992-963724	19921020

AB A method for detecting at least one virulence-assocd. factor (VAF), e.g., a bacterial toxin, in a sample is described. The sample suspected of contg. the VAF-producing bacteria is contacted with a VAF releasing soln. under conditions which release VAF from the bacteria. The released VAF subsequently is immunochem. detected. The preferred method is a membrane-based enzyme linked immunosorbent assay for immunochem. detecting the well-characterized Shiga family toxins including SLT I and SLT II. Also described is the VAF releasing soln. and a kit contg. the reagents for conducting the described methods.

IT **57-13-6, Urea**, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(in virulence-assocd. factor releasing soln.; rapid detection of virulence-assocd. factors)

L8 ANSWER 12 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:129633 HCAPLUS
DOCUMENT NUMBER: 124:173280
TITLE: Invasin of Yersinia pseudotuberculosis activates human peripheral B cells
AUTHOR(S): Lundgren, Erik; Carballeira, Nivia; Vazquez, Raisa; Dubinina, Elena; Braenden, Henrik; Persson, Haekan; Wolf-Watz, Hans
CORPORATE SOURCE: Dep. of Cell and Mol. Biology, Univ. of Umeae, Umeae, S-901 87, Swed.
SOURCE: Infection and Immunity (1996), 64(3), 829-35
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The Yersinia pseudotuberculosis cell surface-located protein invasin was found to promote binding between the pathogen and resting

peripheral B cells via .beta.1 integrin receptors (CD29). B cells responded by expressing several activation markers and by growing. In contrast, T cells did not react, although these cells express CD29. An isogenic invA mutant failed to activate B cells. The mutation could be complemented by providing the invA+ gene in trans. **Purified invasin** alone did not activate B cells, although it was able to block the binding of bacteria to the cells.

L8 ANSWER 13 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:904925 HCAPLUS
DOCUMENT NUMBER: 123:334450
TITLE: Molecular characterization of a carboxy-terminal eukaryotic-cell binding domain of intimin from enteropathogenic Escherichia coli
AUTHOR(S): Frankel, Gad; Candy, David C. A.; Fabiani, Elisa; Adu-Bobie, Jeannette; Gil, Sophie; Novakova, Michaela; Phillips, Alan D.; Dougan, Gordon
CORPORATE SOURCE: Dep. of Biochemistry, Imperial College of Science, London, SW7 2AZ, UK
SOURCE: Infection and Immunity (1995), 63(11), 4323-8
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A eukaryotic cell-binding domain from the intimin (Int) polypeptide of enteropathogenic Escherichia coli O127 (EPEC) was investigated. Derivs. of the carboxy-terminal 280-amino-acid domains of Int (Int-EPEC280) and the Int homolog **invasin** (**Inv**) from Yersinia pseudotuberculosis (InvYP280) were fused to the E. coli maltose-binding protein (MBP), expressed, and **purified** . The smallest MBP-IntEPEC fusion protein that efficiently mediated binding to HEp-2 cells, monitored by using purified fusion proteins in fluorescence activated cell sorter anal. or by using fluorescent Covaspheres coated with purified fusions, contained the carboxy-terminal 150 amino acids of Int. Replacement of Cys-937 with Ser (IntEPEC280CS) destroyed the cell-binding activity of INtEPEC280. Covaspheres coated with MBP-IntEPEC280 were assocd. with HEp-2 cell microvilli but failed to induce actin accumulation underneath bound particles or cell spreading on coated plastic surfaces. MBP-IntEPEC280, but not MBP, MBP-IntEPEC280CS, or MBP-InvYP280, inhibited EPEC entry into HEp-2 cells.

L8 ANSWER 14 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:366334 HCAPLUS
DOCUMENT NUMBER: 122:128483
TITLE: An aspartate residue of the Yersinia pseudotuberculosis invasin protein that is critical for integrin binding
AUTHOR(S): Leong, John M.; Morrissey, Pamela E.; Marra, Andrea; Isberg, Ralph R.
CORPORATE SOURCE: Div. Rheumatol. Immunol., Tufts-New England Med. Center Hosp., Boston, MA, 02111, USA
SOURCE: EMBO Journal (1995), 14(3), 422-31
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Yersinia pseudotuberculosis invasin protein mediates bacterial entry into mammalian cells by binding multiple .beta.1-chain integrins. **Invasin** binding to **purified** .alpha.5.beta.1 integrin is inhibited by Arg-Gly-Asp (RGD)-contg. peptides, although **invasin** contains no RGD sequence. Fifteen mutations that diminished binding and bacterial entry were isolated after mutagenesis of the entire inv gene. All of the mutations altered residues within the C-terminal 192 amino acids of invasin, previously delineated as the integrin binding domain, and 10 of the mutations fell within an 11 residue region. This small region was subjected to site-directed mutagenesis and almost half of the 35 mutations generated decreased invasin-mediated entry. D911 within this region was the most crit. residue, as even a conservative glutamate substitution abolished bacterial penetration. **Purified invasin** derivs. altered at this residue were defective in promoting cell attachment and this defect was reflected in a 10-fold or greater increase in IC50 for integrin binding. D911 may have a function similar to that of the aspartate residue in RGD-contg. sequences.

L8 ANSWER 15 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1994:675712 HCAPLUS
DOCUMENT NUMBER: 121:275712
TITLE: Isolation and identification of eukaryotic receptors promoting bacterial internalization
AUTHOR(S): Van Nhieu, Guy Tran; Isberg, Ralph R.
CORPORATE SOURCE: Department Molecular Biology and Microbiology, Tufts University School Medicine, Boston, MA, 02111, USA
SOURCE: Methods in Enzymology (1994), 236(BACTERIAL PATHOGENESIS, PT. B), 307-18
CODEN: MENZAU; ISSN: 0076-6879
DOCUMENT TYPE: Journal
LANGUAGE: English
AB This article describes the affinity chromatog. technique used to identify members of the integrin family of cell adhesion mols. as cellular receptors for **invasin**, a Yersinia pseudotuberculosis surface protein that allows bacterial internalization, as well as assays allowing the study of the interaction of **invasin** with the **purified** .alpha.5.beta.1-integrin.

L8 ANSWER 16 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:578870 HCAPLUS
DOCUMENT NUMBER: 119:178870
TITLE: The invasin protein of Yersinia spp. provides co-stimulatory activity to human T cells through interaction with .beta.1 integrins
AUTHOR(S): Brett, Sara J.; Mazurov, Alexey V.; Charles, Ian G.; Tite, John P.
CORPORATE SOURCE: Dep. Cell Biol., Wellcome Res. Lab., Beckenham/Kent, BR3 3BS, UK
SOURCE: European Journal of Immunology (1993), 23(7), 1608-14
CODEN: EJIMAF; ISSN: 0014-2980
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The invasin proteins of Yersinia spp. are outer membrane proteins

which are involved in the penetration of these bacteria into mammalian cells. Invasin binds to several different .beta.1 integrins with extremely high affinity, the integrin-binding domain of invasin has been mapped to the C-terminal 192 amino-acids of the mol. Expression of this fragment alone on the cell surface of non-invasive bacteria is enough to confer the invasive phenotype on these strains. Here, the C-terminal 192 amino acids of invasin expressed as a fusion protein with the maltose binding protein of E. coli is capable of delivering co-stimulatory signals to human T cells through the .beta.1 integrins. Co-stimulation was assayed by the ability to **invasin** to augment the response of highly **purified** CD4+ and CD8+ T cells to co-immobilized anti-CD3 antibody. Antibody blocking studies indicated that the co-stimulation was mediated through .beta.1 integrins. The proliferation induced by co-stimulation of CD4+ T cells was accompanied by the synthesis of the cytokines tumor necrosis factor-.alpha. and interferon-.gamma., whereas the activation of CD8+ T cells led to the generation of cytotoxic effectors. The region of the invasin mol. involved in T cell activation was further mapped using synthetic peptides. A region of the invasin mol. contg. the residues TAKSKKFPSY could substitute for invasin in T cell activation. The co-stimulation by peptide could also be inhibited by anti-integrin antibodies. The observation that an outer membrane protein of a bacterium which is assocd. with reactive arthritis and other autoimmune spondyloarthropathies can act as a T cell co-stimulus may have implications for the etiol. of these diseases.

L8 ANSWER 17 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:533771 HCAPLUS
DOCUMENT NUMBER: 119:133771
TITLE: A 76-amino acid disulfide loop in the Yersinia pseudotuberculosis invasin protein is required for integrin receptor recognition
AUTHOR(S): Leong, John M.; Morrissey, Pamela E.; Isberg, Ralph R.
CORPORATE SOURCE: Dep. Med., Tufts-New England Med. Cent. Hosp., Boston, MA, 02111, USA
SOURCE: Journal of Biological Chemistry (1993), 268(27), 20524-32
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Yersinia pseudotuberculosis invasin protein is a 986-amino acid protein that promotes bacterial penetration into mammalian cells by avidly binding multiple .beta.1-chain integrins. A 192-amino acid carboxyl-terminal domain of invasin was previously shown to be sufficient for binding. Evidence is presented here that a 76-amino acid disulfide loop in the integrin binding domain of invasin is required for invasin-mediated cell binding and entry. Bacterial mutants that were altered at either of 2 cysteine residues in the binding domain of invasin were completely defective for entry. **Purified invasin** protein derivs. altered at either of these cysteines, in contrast to the wild-type **invasin**, did not promote either cell binding or penetration. Anal. of proteolytic products of invasin in the presence or absence of reducing agent provided evidence of an intrachain disulfide bond near the carboxyl terminus of the protein. Alkylation of invasin

derivs. with [3H]iodoacetate indicated that these 2 cysteines were normally disulfide-bonded. A treatment that resulted in the maximal redn. of the disulfide bond also resulted in maximal loss of cell attachment activity. These results indicate that the 76-amino acid disulfide loop at the carboxyl terminus of invasin is required for recognition by integrins.

L8 ANSWER 18 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:57714 HCAPLUS
DOCUMENT NUMBER: 118:57714
TITLE: Very late antigen 4-dependent adhesion and costimulation of resting human T cells by the bacterial .beta.1 integrin ligand invasin
AUTHOR(S): Ennis, Elizabeth; Isberg, Ralph R.; Shimizu, Yoji
CORPORATE SOURCE: Med. Sch., Univ. Michigan, Ann Arbor, MI, 48109, USA
SOURCE: Journal of Experimental Medicine (1993), 177(1), 207-12
CODEN: JEMEAV; ISSN: 0022-1007
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bacteria and viruses often use the normal biol. properties of host adhesion mols. to infect relevant host cells. The outer membrane bacterial protein invasin mediates the attachment of Yersinia pseudotuberculosis to human cells. In vitro studies have shown that 4 members of the very late antigen (VLA) integrin family of adhesion mols., VLA-3, VLA-4, VLA-5, and VLA-6, can bind to invasin. Since CD4+ T cells express and use these integrins, the interaction was investigated of CD4+ T cells with **purified invasin**. Although VLA integrin-mediated adhesion of T cells to other ligands such as fibronectin does not occur at high levels unless the T cells are activated, resting T cells bind strongly to **purified invasin**. The binding of resting T cells to invasin requires metabolic activity and an intact cytoskeleton. Although CD4+ T cells express VLA-3, VLA-4, VLA-5, and VLA-6, monoclonal antibody (mAb) blocking studies implicate only VLA-4 as a T cell invasin receptor. Like other integrin ligands, invasin can facilitate T cell proliferative responses induced by a CD3-specific mAb. Thus, the nature of the integrin ligand is a crit. addnl. factor that regulates T cell integrin activity, and direct interactions of T cells with bacterial pathogens such as Yersinia may be relevant to host immune responses to bacterial infection.

L8 ANSWER 19 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1992:38910 HCAPLUS
DOCUMENT NUMBER: 116:38910
TITLE: The **invasin** protein of Yersinia enterocolitica: internalization of **invasin**-bearing bacteria by eukaryotic cells is associated with reorganization of the cytoskeleton
AUTHOR(S): Young, Vincent B.; Falkow, Stanley; Schoolnik, Gary K.
CORPORATE SOURCE: Dep. Microbiol. Immunol., Stanford Univ., Stanford, CA, 94305, USA
SOURCE: Journal of Cell Biology (1992), 116(1), 197-207
CODEN: JCLBA3; ISSN: 0021-9525

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Yersinia enterocolitica, a facultative intracellular pathogen of mammals, readily enters (i.e., invades) cultured eukaryotic cells, a process that can be conferred by the cloned inv locus of the species. This study examd. the mechanism by which the product of **inv**, a microbial outer membrane protein termed "**invasin**", mediates the internalization of bacteria by HEp-2 cells and chicken embryo fibroblasts. **Invasin**-bearing bacteria initially bound the filopodia and the leading edges of cultured cells. Multiple points of contact between the bacterial surface and the surface of the cell ensued and led to the internalization of the bacterium within an endocytic vacuole; the same multistep process could be induced by an inert particle coated with **invasin**-contg. membranes. Both adherence and internalization were blocked by an antisera directed against the .beta.1 integrin cell-adherence mol. Ultrastructural studies of **detergent**-insol. cytoskeletons from infected cells and immunofluorescence microscopy of phalloidin-labeled cells showed alterations in the structure of the cytoskeleton during the internalization process including the accumulation of polymd. actin around entering bacteria. Bacterial entry was prevented by cytochalasin D indicating that the internalization process requires actin microfilament function. Possible linkages between .beta.1 contg. integrins and the cytoskeleton were examd. during the internalization process through the use of protein-specific antibodies and immunofluorescence microscopy. Like actin, the actin-assocd. proteins filamin, talin and the .beta.1 integrin subunit were also found to accumulate around entering bacteria. These findings suggest that the **invasin**-mediated internalization process is assocd. with cytoskeletal reorganization.

L8 ANSWER 20 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:456684 HCAPLUS
DOCUMENT NUMBER: 113:56684
TITLE: Identification of the integrin binding domain of the Yersinia pseudotuberculosis invasin protein
AUTHOR(S): Leong, John M.; Fournier, Robert S.; Isberg, Ralph R.
CORPORATE SOURCE: Sch. Med., Tufts Univ., Boston, MA, 02111, USA
SOURCE: EMBO Journal (1990), 9(6), 1979-89
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The invasin protein of the pathogenic Yersinia pseudotuberculosis mediates entry of the bacterium into cultured mammalian cells by binding several .beta.1 chain integrins. This study identified the region of invasin responsible for cell recognition. Thirty-two monoclonal antibodies directed against invasin were isolated, and of those, six blocked cell attachment to invasin. These six antibodies recognized epitopes within the last 192 amino acids of invasin. Deletion mutants of invasin and maltose-binding protein (MBP)-invasin fusion proteins were generated and tested for cell attachment. All of the invasin derivs. that carried the carboxyl-terminal 192 amino acids retained cell binding activity. One carboxyl-terminal **invasin** fragment and seven MBP-**invasin** fusion proteins were **purified**. The purified derivs. that retained binding activity inhibited bacterial

entry into cultured mammalian cells. These results indicated that the carboxyl-terminal 192 amino acids of invasin contains the integrin-binding domain, even though this region does not contain the tripeptide sequence Arg-Gly-Asp.

L8 ANSWER 21 OF 21 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:196070 HCAPLUS
DOCUMENT NUMBER: 112:196070
TITLE: Multiple .beta.1 chain integrins are receptors for **invasin**, a protein that promotes bacterial penetration into mammalian cells
AUTHOR(S): Isberg, Ralph R.; Leong, John M.
CORPORATE SOURCE: Sch. Med., Tufts Univ., Boston, MA, 02111, USA
SOURCE: Cell (Cambridge, MA, United States) (1990), 60(5), 861-71
CODEN: CELLB5; ISSN: 0092-8674
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mammalian cell receptors that promote entry of intracellular bacteria into nonphagocytic cells have not been identified. In this report, it is shown that multiple members of the integrin superfamily of cell adhesion receptors bind the Yersinia pseudotuberculosis **invasin** protein prior to bacterial penetration into mammalian cells. Affinity chromatog. of crude **detergent** exts. demonstrated that integrins contg. the subunit structures .alpha.3.beta.1, .alpha.4.beta.1, .alpha.5.beta.1, and .alpha.6.beta.1 bound to immobilized **invasin**. Furthermore, phospholipid vesicles contg. isolated integrin proteins were able to attach to **invasin**. Specificity for **invasin** binding to the identified integrin receptors was also demonstrated, as immunoprobing and phospholipid reconstitution studies showed that the .alpha.2.beta.1 integrin, .beta.2 chain integrins, and vitronectin receptor (.alpha.v.beta.3) were not involved in cellular attachment to **invasin**.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER' ENTERED AT 11:44:27 ON 10 JUL 2003)
L9 16 S L6
L10 87 S L7
L11 21 S L10(S)RECOMBINAN?
L12 11 S L10(S)(MANUF? OR PREP? OR PRODUCT? OR PROD##)
L13 41 S L9 OR L11 OR L12
L14 21 DUP REM L13 (20 DUPLICATES REMOVED)

L14 ANSWER 1 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2003:309764 BIOSIS
DOCUMENT NUMBER: PREV200300309764
TITLE: Host cell caveolae act as an entry-port for Group A streptococci.
AUTHOR(S): Rohde, Manfred (1); Mueller, Ellruth; Chhatwal, Gursharan S.; Talay, Susanne R.
CORPORATE SOURCE: (1) Department of Microbial Pathogenicity and Vaccine Research, GBF-German Research Centre for Biotechnology, Mascheroder Weg 1, 38124, Braunschweig, Germany: mro@gbf.de Germany
SOURCE: Cellular Microbiology, (May 2003, 2003) Vol. 5, No. 5, pp. 323-342. print.
ISSN: 1462-5814.

DOCUMENT TYPE: Article
LANGUAGE: English

AB This study identified caveolae as an entry port for group A streptococci into epithelial and endothelial cells. Scanning electron microscopy as well as ultrathin sections of infected cells demonstrated accumulation of small omega-shaped cavities in the host cell membrane close to adherent streptococci. During invasion, invaginations were formed that subsequentely revealed intracellular compartments surrounding streptococci. Caveolin-1 was shown to be present in the membrane of invaginations and the compartment membranes. These compartments were devoid of any classic endosomal/lysosomal marker proteins and can thus be described as caveosomes. Disruption of caveolae with methyl-beta-cyclodextrin and filipin abolished host cell invasion. Importantly, streptococci inside caveosomes avoid fusion with lysosomes. Expressing of Sfbl protein on the surface of the non-invasive S. gordonii resulted in identical morphological alterations on the host cell as for S. pyogenes. Incubation of HUVEC cells with **purified recombinant** sole Sfbl protein also triggered accumulation of cavity-like structures and formation of membrane invaginations. Tagged to colloidal gold-particles, Sfbl protein was shown to cluster following membrane contact. Thus, our results demonstrate that host cell caveolae initiate the invasion process of group A streptococci and that the streptococcal **invasin** Sfbl is the triggering factor that activates the caveolae-mediated endocytic pathway.

L14 ANSWER 2 OF 21 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 2003-328328 [31] WPIDS
CROSS REFERENCE: 2000-072064 [06]; 2001-647179 [74]
DOC. NO. CPI: C2003-085306
TITLE: New nonvirulent bacterium with genes coding for a non-secreted foreign cytolysin or a different foreign agent, useful as an intracellular delivery vehicle for delivering, e.g. vaccines, drugs or genes for therapy to eukaryotic cells.
DERWENT CLASS: B04 D16
INVENTOR(S): HIGGINS, D E; PORTNOY, D A
PATENT ASSIGNEE(S): (HIGG-I) HIGGINS D E; (PORT-I) PORTNOY D A
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2002142007	A1	20021003	(200331)*		14

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 2002142007	A1	Cont of	US 1998-133914	19980813
		Cont of	US 1999-469197	19991221
			US 2001-949109	20010907

PRIORITY APPLN. INFO: US 1998-133914 19980813; US 1999-469197 19991221; US 2001-949109 20010907
AN 2003-328328 [31] WPIDS

CR 2000-072064 [06]; 2001-647179 [74]
AB US2002142007 A UPAB: 20030516
NOVELTY - A nonvirulent bacterium, which comprises a first gene encoding a non-secreted foreign cytolysin operably linked to a heterologous promoter and a second gene encoding a different foreign agent, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) A eukaryotic cell comprising the nonvirulent bacterium, and further comprising the foreign cytolysin; and

(2) Introducing a foreign agent into a eukaryotic cell by contacting the cell with the nonvirulent bacterium such that the agent enters the cell.

USE - The nonvirulent bacterium is useful as an intracellular delivery vehicle, particularly of agents to eukaryotic cells. The nonvirulent bacterium is particularly useful for delivering foreign agents for diagnosis, therapy (e.g. prophylactics such as vaccine, delivery of therapeutic drug, or gene therapy), or biosynthesis. The nonvirulent bacterium is also useful for delivering nucleic acids that provide templates for transcription or translation, or provide modulators of transcription and/or translation.

ADVANTAGE - No protein **purification** is required compared to prior art delivery systems. In addition, high levels of protein can be delivered to the cytosol of virtually any cell and the levels can be controlled through the use of inducible promoters. Dwg.0/2

L14 ANSWER 3 OF 21 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002622038 MEDLINE
DOCUMENT NUMBER: 22267124 PubMed ID: 12379706
TITLE: Intracellular growth of Legionella pneumophila gives rise to a differentiated form dissimilar to stationary-phase forms.
AUTHOR: Garduno Rafael A; Garduno Elizabeth; Hiltz Margot; Hoffman Paul S
CORPORATE SOURCE: Department of Microbiology and Immunology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H-4H7.
SOURCE: INFECTION AND IMMUNITY, (2002 Nov) 70 (11) 6273-83. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20021017
Last Updated on STN: 20021213
Entered Medline: 20021108

AB When Legionella pneumophila grows in HeLa cells, it alternates between a replicative form and a morphologically distinct "cyst-like" form termed MIF (mature intracellular form). MIFs are also formed in natural amoebic hosts and to a lesser extent in macrophages, but they do not develop in vitro. Since MIFs accumulate at the end of each growth cycle, we investigated the possibility that they are in vivo equivalents of stationary-phase (SP) bacteria, which are enriched for virulence traits. By electron microscopy, MIFs appeared as short, stubby rods with an electron-dense, laminar outer membrane layer and a cytoplasm largely

occupied by inclusions of poly-beta-hydroxybutyrate and laminations of internal membranes originating from the cytoplasmic membrane. These features may be responsible for the bright red appearance of MIFs by light microscopy following staining with the phenolic Gimenez stain. In contrast, SP bacteria appeared as dull red rods after Gimenez staining and displayed a typical gram-negative cell wall ultrastructure. Outer membranes from MIFs and SP bacteria were equivalent in terms of the content of the peptidoglycan-bound and disulfide bond cross-linked OmpS porin, although additional proteins, including Hsp60 (which acts as an **invasin** for HeLa cells), were detected only in preparations from MIFs. Proteomic analysis revealed differences between MIFs and SP forms; in particular, MIFs were enriched for an approximately 20-kDa protein, a potential marker of development. Compared with SP bacteria, MIFs were 10-fold more infectious by plaque assay, displayed increased resistance to rifampin (3- to 5-fold) and gentamicin (10- to 1,000-fold), resisted **detergent** -mediated lysis, and tolerated high pH. Finally, MIFs had a very low respiration rate, consistent with a decreased metabolic activity. Collectively, these results suggest that intracellular L. pneumophila differentiates into a cyst-like, environmentally resilient, highly infectious, post-SP form that is distinct from in vitro SP bacteria. Therefore, MIFs may represent the transmissible environmental forms associated with Legionnaires' disease.

L14 ANSWER 4 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:584856 BIOSIS
DOCUMENT NUMBER: PREV200200584856
TITLE: The AggB and Agg3B proteins produced by enteroaggregative Escherichia coli are two distinct invasins of the AfaD family.
AUTHOR(S): Bernier, C. (1); Le Bouguenec, C. C. (1)
CORPORATE SOURCE: (1) Institut Pasteur, Paris France
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 64. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology Salt Lake City, UT, USA May 19-23, 2002 American Society for Microbiology . ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Enteroaggregative Escherichia coli (EAEC) strains cause intestinal disorders. These bacteria represent an emerging pathotype of increasing importance. EAEC are involved in acute and persistent diarrhea, in food-borne diarrhea out-breaks and in traveler's diarrhea. We characterized the AAF-III system (agg3 operon) from strain 55989 isolated from an HIV positive patient suffering from persistent diarrhea. Analysis of the sequence of agg3 showed a genetic organization very similar to agg and aaf operons encoding, respectively, AAF-I and AAF-II fimbriae, and to afa operons encoding afimbrial adhesins. Significant similarities were found between the Agg3B protein and members of the AfaD family of **invasins**, with AfaD-III (from the afa-3 operon) as a prototype. Polystyrene beads coated with **purified recombinant** AggB and Agg3B proteins were internalized in HeLa cells while AafB-coated beads were not. AfaD-III was used as a positive control. We

previously demonstrated that AfaD-III interacts with cell surface proteins like the a5b1 integrin. To test the hypothesis that the **invasins** produced by EAEC interact with the same receptor, solubilized HeLa proteins were separated by SDS-PAGE and transferred onto nitrocellulose filters. These were then overlayed with AggB, AafB and Agg3B proteins. As for AfaD-III, we demonstrated binding of AggB and Agg3B to cellular protein corresponding to a5b1 integrin. No binding of AafB to cellular proteins was demonstrated. Previous reports concerning EAEC isolates demonstrated that AAF-I, but not AAF-II strains, were internalized in intestinal cells. We also demonstrated that strains producing the AAf-III fimbriae invade HeLa cells. The ability of EAEC strains to invade epithelial cells may reflect an evolutionnary strategy designed to establish bacterial reservoirs and persistence within the host.

L14 ANSWER 5 OF 21 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001420191 MEDLINE
DOCUMENT NUMBER: 21360980 PubMed ID: 11468407
TITLE: Expression, refolding and crystallization of the OpcA **invasin** from Neisseria meningitidis.
AUTHOR: Prince S M; Feron C; Janssens D; Lobet Y; Achtman M; Kusecek B; Bullough P A; Derrick J P
CORPORATE SOURCE: Department of Biomolecular Sciences, UMIST, PO Box 88, Manchester, England.
SOURCE: ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (2001 Aug) 57 (Pt 8) 1164-6. Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20011008
Last Updated on STN: 20011008
Entered Medline: 20011004

AB OpcA is an integral outer membrane from the Gram-negative pathogen Neisseria meningitidis that plays a role in adhesion of meningococci to host cells. The protein was overexpressed in Escherichia coli in an insoluble form and a procedure developed for refolding by rapid dilution from **denaturant** into **detergent** solution. The refolded material was identical to native OpcA isolated from meningococci, as judged by overall molecular weight, migration on SDS-PAGE and reaction against monoclonal antibodies. Both native and recombinant OpcA crystallized under similar conditions to give an orthorhombic crystal form (P2(1)2(1)2), with unit-cell parameters a = 96.9, b = 46.3, c = 74.0 A. Complete data sets of reflections were collected from native and refolded OpcA to 2.0 A resolution.

L14 ANSWER 6 OF 21 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2002092648 MEDLINE
DOCUMENT NUMBER: 21580459 PubMed ID: 11722186
TITLE: Cloning, expression, and purification of the uropathogenic Escherichia coli invasin DraD.
AUTHOR: Zalewska B; Piatek R; Cieslinski H; Nowicki B; Kur J
CORPORATE SOURCE: Department of Microbiology, Technical University of Gdansk, ul. Narutowicza 11/12, Gdansk, 80-952, Poland.

CONTRACT NUMBER: DK-42029 (NIDDK)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (2001 Dec) 23 (3) 476-82.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020202
Last Updated on STN: 20020501
Entered Medline: 20020430

AB In this study we presented a very efficient expression system, based on pET30LIC/Ek vector, for producing DraD **invasin** of the uropathogenic Escherichia coli and a one-step chromatography **purification** procedure for obtaining **pure recombinant** protein (DraD-C-His(6)). This protein has a molecular weight of 14,818 and calculated pI of 6.6. It contains a polyhistidine tag at the C-terminus (13 additional amino acids) that allowed single-step isolation by Ni affinity chromatography. Also, we obtained specific antibodies against DraD invasin to develop tools for characterizing the expression and biological function of this protein. The amount and quality of DraD-C-His(6) fusion protein purified from E. coli overexpression system seems to be fully appropriate for crystallographic studies (soluble form), and for establishing role of the protein in bacterium (cultured cell line interaction and in the internalization process) and for obtaining rabbit polyclonal antisera (insoluble form).

L14 ANSWER 7 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 4

ACCESSION NUMBER: 2001:72726 BIOSIS
DOCUMENT NUMBER: PREV200100072726
TITLE: Enterotoxigenic Escherichia coli TibA glycoprotein adheres to human intestine epithelial cells.
AUTHOR(S): Lindenthal, Christoph; Elsinghorst, Eric A. (1)
CORPORATE SOURCE: (1) Department of Molecular Biosciences, University of Kansas, 7049 Haworth Hall, Lawrence, KS, 66045-2106: elsingh@ukans.edu USA
SOURCE: Infection and Immunity, (January, 2001) Vol. 69, No. 1, pp. 52-57. print.
ISSN: 0019-9567.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Enterotoxigenic Escherichia coli (ETEC) is capable of invading epithelial cell lines derived from the human ileum and colon. Two separate invasion loci (tia and tib) that direct noninvasive E. coli strains to adhere to and invade cultured human intestine epithelial cells have previously been isolated from the classical ETEC strain H10407. The tib locus directs the synthesis of TibA, a 104-kDa outer membrane glycoprotein. Synthesis of TibA is directly correlated with the adherence and invasion phenotypes of the tib locus, suggesting that this protein is an adhesin and **invasin**. Here we report the **purification** of TibA and characterization of its biological activity. TibA was **purified** by continuous-elution **preparative** sodium dodecyl

sulfate-polyacrylamide gel electrophoresis. **Purified** TibA was biotin labeled and then shown to bind to HCT8 human ileocecal epithelial cells in a specific and saturable manner. Unlabeled TibA competed with biotin-labeled TibA, suggesting the presence of a specific TibA receptor in HCT8 cells. These results show that TibA acts as an adhesin. Polyclonal anti-TibA antiserum inhibited invasion of ETEC strain H10407 and of **recombinant** E. coli bearing tib locus clones, suggesting that TibA also acts as an **invasin**. The ability of TibA to direct epithelial cell adhesion suggests a role for this protein in ETEC pathogenesis.

L14 ANSWER 8 OF 21 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 5
ACCESSION NUMBER: 2000-339646 [29] WPIDS
DOC. NO. CPI: C2000-103099
TITLE: New **purified recombinant** **invasin** proteins IpaC and SipC useful as an adjuvant and vaccine against shigellosis, salmonellosis and enteroinvasive Escherichia coli.
DERWENT CLASS: B04 D16
INVENTOR(S): OAKS, E V; PICKING, W D
PATENT ASSIGNEE(S): (UYSL-N) UNIV ST LOUIS
COUNTRY COUNT: 90
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000023462	A1	20000427	(200029)*	EN	78
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000012277	A	20000508	(200037)		
EP 1131338	A1	20010912	(200155)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000023462	A1	WO 1999-US24931	19991021
AU 2000012277	A	AU 2000-12277	19991021
EP 1131338	A1	EP 1999-970664	19991021
		WO 1999-US24931	19991021

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000012277	A	Based on	WO 200023462
EP 1131338	A1	Based on	WO 200023462

PRIORITY APPLN. INFO: US 1999-136754P 19990601; US 1998-105085P 19981021
AN 2000-339646 [29] WPIDS
AB WO 200023462 A UPAB: 20000617

NOVELTY - A composition comprising, **recombinant invasin** protein (I), of at least 95% purity, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method for producing a **purified invasin** protein comprising:

(a) inserting a polynucleotide encoding an **invasin** protein into an expression vector, or combining a polynucleotide encoding the **invasin** protein and a polynucleotide encoding an affinity **purification** moiety;

(b) transforming the combination of (a) into a host cell;

(c) growing the host cell for the expression soluble protein;

(d) extracting the protein from a host cell lysate, culture medium, or reconstituted organism with a solution comprising a protein **denaturant**;

(e) performing an affinity **purification** of the **invasin** protein in the presence of a protein **denaturant**;

(f) removing the protein **denaturant** from the solution obtained from (e), until the concentration of the **denaturant** is at the minimum concentration necessary to maintain protein solubility; and

(g) rapidly diluting the **purified** protein into a volume of **denaturant**-free solution;

(2) an adjuvant composition comprising at least one **purified recombinant invasin** protein, where administration in combination with an antigen elicits an immune response to the antigen;

(3) an adjuvant composition, comprising a **recombinant invasin** protein, of at least 95% purity, and having adjuvant activity, the **invasin** protein comprising an amino acid sequence derived from a protein of a member of the Shigella or Salmonella genus, or enteroinvasive Escherichia coli, administration of the composition, in combination with an antigen, to an animal results in the **production**, by Th2 cells, of at least one cytokine, selected from interleukin (IL)-4, 5, 6, 10, and 13, or in the **production** of at least one immunoglobulin selected from IgG, IgE, IgM, and IgA;

(4) a vaccine **preparation** comprising a **purified recombinant invasin** protein having adjuvant activity, at least one antigen, and a carrier, diluent or excipient;

(5) a vaccine **preparation**, comprising a **purified invasin** protein of at least 95% purity and having adjuvant activity, the protein comprises an amino acid sequence derived from a protein of a member of the Shigella or Salmonella genus, or from an enteroinvasive E. coli, at least one antigen, and a diluent, carrier, or excipient;

(6) a method for eliciting an immune response in an animal by administering an adjuvant composition comprising a **purified recombinant invasin** protein;

(7) methods for stimulating the **production** of at least one cytokine, selected from IL-4, 5, 6, 10, and 13, by Th2 cells, comprising administering a **purified recombinant invasin** protein of at least 95% purity, comprising an amino acid sequence derived from a protein of a member of the Shigella or Salmonella genus, or from a enteroinvasive E. coli;

(8) a method for stimulating **production** of at least one class of immunoglobulin, selected from IgG, IgE, IgM, and IgA, comprising administering a **purified recombinant invasin** protein of at least 95% purity, comprising an amino acid sequence derived from a protein of a member of the Shigella or Salmonella genus, or from a enteroinvasive E. coli; and

(9) methods for delivering pharmacologically active, therapeutic, cytotoxic or diagnostic substances into cells by administering a pharmacologically active, therapeutic, cytotoxic or diagnostic substance and a **recombinant invasin** protein or a fusion protein comprising a **recombinant invasin** protein.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Vaccine.

USE - (I) may be used as a vaccine and as an adjuvant for the prevention of diseases such as shigellosis, salmonellosis and diseases caused by enteroinvasive E. coli, and in stimulating the immune system of immuno-compromised individuals (claimed). (I) is also useful for intracellular delivery of therapeutic and diagnostic agents, and to stimulate immune response by cells in vitro. (I) can be mixed with antigens of biological or chemical origins to elicit an immune response to the antigen.

ADVANTAGE - The new method allows the **production** of fully soluble, biologically active invasion proteins, which are substantially free of **denaturants**, and are at least 95% **pure**. The new **invasin** proteins are superior to presently approved adjuvants due to their low toxicity, their ability to stimulate both peripherals and mucosal immune response, and ease of **production**.

Dwg.0/6

L14 ANSWER 9 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 6

ACCESSION NUMBER: 2002:440006 BIOSIS
DOCUMENT NUMBER: PREV200200440006
TITLE: Epithelial cell adherence mediated by the enterotoxigenic Escherichia coli Tia protein.
AUTHOR(S): Mammarappallil, Joseph G.; Elsinghorst, Eric A. (1)
CORPORATE SOURCE: (1) Department of Molecular Biosciences, University of Kansas, 7049 Haworth Hall, Lawrence, KS, 66045-2106: elsingh@ukans.edu USA
SOURCE: Infection and Immunity, (December, 2000) Vol. 68, No. 12, pp. 6595-6601. print.
ISSN: 0019-9567.
DOCUMENT TYPE: Article
LANGUAGE: English

AB In vitro studies have shown that enterotoxigenic Escherichia coli (ETEC) strains are capable of invading cultured epithelial cells derived from the human ileum and colon. Two separate invasion loci (tia and tib) have previously been isolated from the classical ETEC strain H10407. The tia locus has been shown to direct the synthesis of Tia, a 25-kDa outer membrane protein. Tia is sufficient to confer the adherence and invasion phenotypes on laboratory stains of E. coli, suggesting that this protein is an adhesin and **invasin**. Here we report the **purification** of Tia and characterize its biological activity. Tia was **purified** by electroelution of outer membrane proteins that had been separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Purified Tia was labeled with biotin and then shown to bind to HCT8 human ileocecal epithelial cells in a specific and saturable manner. Polyclonal anti-Tia antiserum blocked this binding. These results show that Tia acts as an adhesin. Polyclonal anti-Tia antiserum also inhibited invasion of **recombinant** E. coli bearing tia clones, indirectly suggesting that Tia may also act as an **invasin**. We prediict Tia to contain eight transmembrane amphiphatic beta-sheets with four loops that are exposed on the surface of the bacterial cell. A peptide corresponding to 19 residues in one of the four predicted surface-exposed loops inhibits Tia-mediated epithelial cell invasion. Seeding HCT8 cells on wells coated with **purified** Tia reduced Tia-mediated epithelial cell invasion. Together, these results indicate that Tia is an **invasin** and adhesin that binds a specific receptor on HCT8 cells.

L14 ANSWER 10 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 7

ACCESSION NUMBER: 2000:439640 BIOSIS
DOCUMENT NUMBER: PREV200000439640
TITLE: A multi-domain protein for beta1 integrin-targeted DNA delivery.
AUTHOR(S): Fortunati, E.; Ehlert, E.; van Loo, N.-D.; Wyman, C.; Eble, J. A.; Grosveld, F.; Scholte, B. J. (1)
CORPORATE SOURCE: (1) Dept Cell Biology and Genetics, Erasmus University, 3000 DR, Rotterdam Netherlands
SOURCE: Gene Therapy, (September, 2000) Vol. 7, No. 17, pp. 1505-1515. print.
ISSN: 0969-7128.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The development of effective receptor-targeted nonviral vectors for use in vivo is complicated by a number of technical problems. One of these is the low efficiency of the conjugation procedures used to couple protein ligands to the DNA condensing carrier molecules. We have made and characterized a multi-domain protein (SPKR)4inv, that is designed to target plasmid DNA to beta1 integrins in remodeling tissue. It contains a nonspecific DNA-binding domain (SPKR)4, a rigid alpha-helical linker, and the C-terminal beta1 integrin binding domain (aa 793-987) of the Yersinia pseudotuberculosis **invasin** protein. (SPKR)4inv could be **purified** at high yields using a bacterial expression system. We show that (SPKR)4inv binds with high affinity to both plasmid DNA and beta1 integrins. In a cell attachment assay, the apparent affinity of (SPKR)4inv for beta1 integrins is three orders of magnitude higher than that of the synthetic peptide integrin ligand RGDS. (SPKR)4inv-plasmid complexes are not active in an in vitro transfection assay. However, transfection efficiencies of plasmid complexes with a cationic lipid micelle (DOTAP/Tween-20) or a cationic polymer (polyethylenimine), are significantly increased in combination with (SPKR)4inv. (SPKR)4inv-mediated transfection can be inhibited by a soluble form of beta1 integrin, which is evidence for its receptor specificity. In conclusion, (SPKR)4inv allows beta1 integrin-specific targeting of plasmid-carrier complexes, while avoiding inefficient and cumbersome coupling chemistry. The modular design of the expression vector allows **production** of similar multi-domain proteins with a different affinity. The further

development of such complexes for use in vivo is discussed.

L14 ANSWER 11 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 8
ACCESSION NUMBER: 2000:956 BIOSIS
DOCUMENT NUMBER: PREV200000000956
TITLE: Preincubation of recombinant Ipa proteins of Shigella sonnei promotes entry of non-invasive Escherichia coli into HeLa cells.
AUTHOR(S): Terajima, Jun; Moriishi, Eiko; Kurata, Takeshi; Watanabe, Haruo (1)
CORPORATE SOURCE: (1) Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku, Tokyo, 162-8640 Japan
SOURCE: Microbial Pathogenesis, (Oct., 1999) Vol. 27, No. 4, pp. 223-230.
ISSN: 0882-4010.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Invasion plasmid antigens of Shigella sonnei, IpaB, C, D, were expressed as fusion proteins either with maltose-binding protein (MBP) or Strept-tag sequence. Affinity-**purified** IpaB and IpaD were separated from MBP by digestion with Factor Xa. **Recombinant** IpaC having Strept-tag sequence at its C-terminal was also **purified** by avidin affinity column chromatography. These **recombinant** proteins showed the ability to cause non-invasive Escherichia coli K-12 to internalize HeLa cell only when all of the proteins were preincubated with the bacterial prior to the inoculation of the mixture into HeLa cell culture. Electron microscopy also showed internalized bacteria within HeLa cells suggesting that functional complex of **invasins** (IpaB, C and D) were formed in vitro.

L14 ANSWER 12 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 9
ACCESSION NUMBER: 1999:526161 BIOSIS
DOCUMENT NUMBER: PREV199900526161
TITLE: The Tir-binding region of enterohaemorrhagic Escherichia coli intimin is sufficient to trigger actin condensation after bacterial-induced host cell signalling.
AUTHOR(S): Liu, Hui; Magoun, Loranne; Luperchio, Steve; Schauer, David B.; Leong, John M. (1)
CORPORATE SOURCE: (1) Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA, 01655 USA
SOURCE: Molecular Microbiology, (Oct., 1999) Vol. 34, No. 1, pp. 67-81.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English.
AB Enterohaemorrhagic Escherichia coli (EHEC) has emerged as an important agent of diarrhoeal disease. Attachment to host cells, an essential step during intestinal colonization by EHEC, is associated with the formation of a highly organized cytoskeletal structure

containing filamentous actin, termed an attaching and effacing (A/E) lesion, directly beneath bound bacteria. The outer membrane protein intimin is required for the formation of this structure, as is Tir, a bacterial protein that is translocated into the host cell and is thought to function as a receptor for intimin. To understand intimin function better, we fused EHEC intimin to a homologous protein, Yersinia pseudotuberculosis **invasin**, or to maltose-binding protein. The N-terminal 539 amino acids of intimin were sufficient to promote outer membrane localization of the C-terminus of **invasin** and, conversely, the N-terminal 489 amino acids of **invasin** were sufficient to promote the localization of the C-terminus of intimin. The C-terminal 181 residues of intimin were sufficient to bind mammalian cells that had been preinfected with an enteropathogenic E. coli strain that expresses Tir but not intimin. Binding of intimin derivatives to preinfected cells correlated with binding to **recombinant** Tir protein. Finally, the 181-residue minimal Tir-binding region of intimin, when **purified** and immobilized on latex beads, was sufficient to trigger A/E lesions on preinfected mammalian cells.

L14 ANSWER 13 OF 21 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 1998359768 MEDLINE
DOCUMENT NUMBER: 98359768 PubMed ID: 9692987
TITLE: **Recombinant** soluble human alpha 3 beta 1 integrin: **purification**, processing, regulation, and specific binding to laminin-5 and **invasin** in a mutually exclusive manner.
AUTHOR: Eble J A; Wucherpfennig K W; Gauthier L; Dersch P; Krukonis E; Isberg R R; Hemler M E
CORPORATE SOURCE: Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA..
eble@uni-muenster.de
CONTRACT NUMBER: CA42368 (NCI)
SOURCE: BIOCHEMISTRY, (1998 Aug 4) 37 (31) 10945-55.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980903
Last Updated on STN: 19980903
Entered Medline: 19980824

AB Using insect cells, we expressed large quantities of soluble human integrin alpha 3 beta 1 ectodomain heterodimers, in which cytoplasmic and transmembrane domains were replaced by Fos and Jun dimerization motifs. In direct ligand binding assays, soluble alpha 3 beta 1 specifically bound to laminin-5 and laminin-10, but not to laminin-1, laminin-2, fibronectin, various collagens, nidogen, thrombospondin, or complement factors C3 and C3b. Soluble alpha 3 beta1 integrin also bound to invasin, a bacterial surface protein, that mediates entry of Yersinia species into the eukaryotic host cell. Invasin completely displaced laminin-5 from the alpha 3 beta 1 integrin, suggesting sterically overlapping or identical binding sites. In the presence of 2 mM Mg2+, alpha 3 beta 1's binding affinity for invasin (Kd = 3.1 nM) was substantially greater than its affinity for laminin-5 (Kd > 600 nM). Upon addition of 1 mM Mn2+, or activating antibody 9EG7, binding affinity for both

laminin-5 and invasin increased by about 10-fold, whereas the affinity decreased upon addition of 2 mM Ca2+. Thus, functional regulation of the purified soluble integrin alpha 3 beta 1 ectodomain heterodimer resembles that of wild-type membrane-anchored beta 1 integrins. The integrin alpha 3 subunit was entirely cleaved into disulfide-linked heavy and light chains, at a newly defined cleavage site located C-terminal of a tetrabasic RRRR motif. Within the alpha 3 light chain, all potential N-glycosylation sites bear N-linked mannose-rich carbohydrate chains, suggesting an important structural role of these sugar residues in the stalk-like region of the integrin heterodimer. In conclusion, studies of our recombinant alpha 3 beta 1 integrin have provided new insights into alpha 3 beta1 structure, ligand binding function, specificity, and regulation.

L14 ANSWER 14 OF 21 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97283291 EMBASE
DOCUMENT NUMBER: 1997283291
TITLE: Gene transfer using a novel fusion protein, GAL4/Invasin.
AUTHOR: Paul R.W.; Weisser K.E.; Loomis A.; Sloane D.L.; LaFoe D.; Atkinson E.M.; Overell R.W.
CORPORATE SOURCE: Dr. R.W. Paul, Department of Molecular Biology, Targeted Genetics Corporation, 1100 Olive Way, Seattle, WA 98101, United States
SOURCE: Human Gene Therapy, (1997) 8/10 (1253-1262). Refs: 51
ISSN: 1043-0342 CODEN: HGTHE3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The delivery of DNA to target cells using simple, defined, nonviral systems has become an area of intense interest in gene therapy. We describe here the development and characterization of one such novel system. A **recombinant**, bifunctional, fusion protein was expressed and **purified** from Escherichia coli. This protein consists of the DNA-binding domain of the yeast transcription factor GAL4 fused to the cell binding, internalization domain of the Yersinia pseudotuberculosis **inv** gene **product**, **invasin**. This protein, GAL4/**Inv**, together with poly-L-lysine, formed complexes with a chloramphenicol acetyltransferase (CAT) reporter plasmid that contains eight repeats of the GAL4 consensus recognition sequence. These complexes were shown to transfect target cells in an **invasin** receptor-dependent manner, resulting in transient CAT expression. A simple, targeted DNA delivery vehicle, as we describe here, represents a viable approach to nonviral gene delivery.

L14 ANSWER 15 OF 21 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 1996-412060 [41] WPIDS
DOC. NO. NON-CPI: N1996-346898
DOC. NO. CPI: C1996-129846
TITLE: Detection of bacterial virulence-associated factor in faeces - by immunoassay after release with soln. contg. surfactant, **urea** and antibiotic.
DERWENT CLASS: A96 B04 D16 S03

INVENTOR(S): THORNE, G M
PATENT ASSIGNEE(S): (CHIL-N) CHILDRENS MEDICAL CENT
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5552294	A	19960903	(199641)*		9

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 5552294	A	Cont of	US 1992-963724	19921020
			US 1994-279832	19940725

PRIORITY APPLN. INFO: US 1992-963724 19921020; US 1994-279832 19940725
AN 1996-412060 [41] WPIDS
AB US 5552294 A UPAB: 19961011
Method for detecting a virulence-associated factor (VAF) in a faecal sample comprises:
(a) treating the sample with a VAF-releasing soln. to release 1 VAF from any VAF-producing bacteria in the sample and
(b) immunochemically detecting the presence or amt. of released VAF.
The VAF is a bacterial toxin, surface antigen, adhesive factor or heat-release protein. The VAF-releasing soln. contains a surfactant, **urea** and an antibiotic selected from polymyxins and mitomycin C.
USE - The method is used esp. for detecting Shiga-like toxin I (SLT I) and/or Shiga-like toxin II (SLT II), heat-labile enterotoxin, heat stabile enterotoxins a+b, heat-stabile-like enterotoxins, adhesins, lipopolysaccharide-0157 antigen, haemolysin, cholera toxin, flagella, zot toxin, toxin A, toxin B, surface antigen for invasion, cytotoxins, proteases, siderophores, **invasins**, outer membrane protein, pili and lipopolysaccharides (all claimed).
ADVANTAGE - Bacterial toxins are released without destroying their structure and without interfering with the immunoassay.
Dwg.0/3

L14 ANSWER 16 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 11
ACCESSION NUMBER: 1997:66032 BIOSIS
DOCUMENT NUMBER: PREV199799365235
TITLE: A pathogen-specific epitope inserted into recombinant secretory immunoglobulin A is immunogenic by the oral route.
AUTHOR(S): Corthesy, Blaise (1); Kaufmann, Muriel; Phalipon, Armelle; Peitsch, Manuel; Neutra, Marian R.; Kraehenbuhl, Jean-Pierre
CORPORATE SOURCE: (1) Institut Suisse de Recherches Experimentales sur le Cancer et Institut de Biochimie, Chemin des Boveresses 155, CH-1066 Epalinges Switzerland
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 52, pp. 33670-33677.

ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Oral administration of rabbit secretory IgA (sIgA) to adult BALB/c mice induced IgA+, IgM+, and IgG+ lymphoblasts in the Peyer's patches, whose fusion with myeloma cells resulted in hybridomas producing IgA, IgM, and IgG1 antibodies to the secretory component (SC). This suggests that SC could serve as a vector to target protective epitopes into mucosal lymphoid tissue and elicit an immune response. We tested this concept by inserting a Shigella flexneri **invasin** B epitope into SC, which, following reassociation with IgA, was delivered orally to mice. To identify potential insertion sites at the surface of SC, we constructed a molecular model of the first and second Ig-like domains of rabbit SC. A surface epitope recognized by an SC-specific antibody was mapped to the loop connecting the E and F beta strands of domain I. This 8-amino acid sequence was replaced by a 9-amino acid linear epitope from S. flexneri **invasin** B. We found that cellular trafficking of **recombinant** SC produced in mammalian CV-1 cells was drastically altered and resulted in a 50-fold lower rate of secretion. However, **purification** of chimeric SC could be achieved by Ni-2+-chelate affinity chromatography. Both wild-type and chimeric SC bound to dimeric IgA, but not to monomeric IgA. Reconstituted sIgA carrying the **invasin** B epitope within the SC moiety triggers the appearance of seric and salivary **invasin** B-specific antibodies. Thus, neo-antigenized sIgA can serve as a mucosal vaccine delivery system inducing systemic and mucosal immune responses.

L14 ANSWER 17 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1997:37658 BIOSIS
DOCUMENT NUMBER: PREV199799329646
TITLE: Cloning, expression, and affinity purification of recombinant Shigella flexneri invasion plasmid antigens IpaB and IpaC.
AUTHOR(S): Picking, Wendy L.; Mertz, Jennifer A.; Marquart, Mary E.; Picking, William D. (1)
CORPORATE SOURCE: (1) Saint Louis Univ., Dep. Biol., 3507 Laclede Avenue, St. Louis, MO 63103-2010 USA
SOURCE: Protein Expression and Purification, (1996) Vol. 8, No. 4, pp. 401-408.
ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Shigella flexneri and related enteropathogenic bacteria are important agents of bacillary dysentery, a potentially life-threatening illness for children in underdeveloped regions of the world. Onset of shigellosis stems from S. flexneri invasion of colonic epithelial cells, leading to localized cell death and inflammation. Invasion plasmid antigens (Ipa) B, C, and D are three secreted proteins encoded by the large virulence plasmid of S. flexneri that have been implicated as essential effectors of this cell invasion process. These proteins are expressed as part of the ipa operon and are among the major targets of the host immune response to shigellosis. Biochemical characterization of the Ipa **invasins** has been complicated by the fact they have not been **purified** in the quantities needed for detailed in vitro analysis. Here we describe the first cloning, expression, and

extensive **purification** of IpaB and IpaC fusion proteins from Escherichia coli for use in dissecting of the protein biochemistry of S. flexneri pathogenesis. A variety of approaches were used to **prepare** significant quantities of these proteins in their soluble forms, including the use of different host cell lines, modification of bacterial growth conditions, and the use of alternative plasmid expression vectors. Now that these Ipa proteins are available in a highly **pure** form, it will be possible to initiate studies on their important biological and immunological properties as well as their recruitment into high-molecular-weight protein complexes. Together with IpaD (**purified** as part of a previous study), these **purified** proteins will be useful for: (a) exploring properties of the host immune response to S. flexneri invasion, (b) elucidating the specific biochemical properties that lead to pathogen internalization, (c) analyzing the importance of specific Ipa protein complexes in host cell invasion, and (d) monitoring, or perhaps even augmenting, the efficacy of live oral vaccines in human trials.

L14 ANSWER 18 OF 21 MEDLINE
ACCESSION NUMBER: 95369928 MEDLINE
DOCUMENT NUMBER: 95369928 PubMed ID: 7642302
TITLE: Increased protein secretion and adherence to HeLa cells by Shigella spp. following growth in the presence of bile salts.
AUTHOR: Pope L M; Reed K E; Payne S M
CORPORATE SOURCE: Department of Microbiology, University of Texas at Austin 78712, USA.
SOURCE: INFECTION AND IMMUNITY, (1995 Sep) 63 (9) 3642-8. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950930
Last Updated on STN: 19970203
Entered Medline: 19950921

AB Growth of Shigella spp. in the presence of the bile salt deoxycholate or chenodeoxycholate enhanced the bacterial invasion of HeLa cells. Growth in the presence of other structurally similar bile salts or **detergents** had little or no effect. Deoxycholate-enhanced invasion was not observed when bacteria were exposed to deoxycholate at low temperatures or when chloramphenicol was added to the growth medium, indicating that bacterial growth and protein synthesis are required. Increased invasion is associated with the presence of an intact Shigella virulence plasmid and is correlated with increased secretion of a set of proteins, including the Ipa proteins, to the outer membrane and into the growth medium. The increased invasion induced by the bile salts appears to be due to increased adherence. The enhanced adherence was specific to Shigella spp., since the enteroinvasive Escherichia coli strains tested did not exhibit the effect in response to growth in bile salts.

L14 ANSWER 19 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 12

ACCESSION NUMBER: 1993:501158 BIOSIS
DOCUMENT NUMBER: PREV199396125165
TITLE: A 76-amino acid disulfide loop in the Yersinia pseudotuberculosis invasin protein is required for integrin receptor recognition.
AUTHOR(S): Leong, John M.; Morrissey, Pamela E.; Isberg, Ralph R. (1)
CORPORATE SOURCE: (1) Howard Hughes Med. Inst. Dep. Mol. Biol. Microbiol., Tufts Univ. Sch. Med., 136 Harrison Ave., Boston, MA 02111 USA
SOURCE: Journal of Biological Chemistry, (1993) Vol. 268, No. 27, pp. 20524-20532.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The Yersinia pseudotuberculosis **invasin** protein is a 986-amino acid protein that promotes bacterial penetration into mammalian cells by avidly binding multiple beta-1-chain integrins. A 192-amino acid carboxyl-terminal domain of **invasin** was previously shown to be sufficient for binding. Evidence is presented here that a 76-amino acid disulfide loop in the integrin binding domain of **invasin** is required for **invasin** -mediated cell binding and entry. Bacterial mutants that were altered at either of 2 cysteine residues in the binding domain of **invasin** were completely defective for entry. **Purified invasin** protein derivatives altered at either of these cysteines, in contrast to the wild-type **invasin**, did not promote either cell binding or penetration. Analysis of proteolytic **products** of **invasin** in the presence or absence of reducing agent provided evidence of an intrachain disulfide bond near the carboxyl terminus of the protein. Alkylation of **invasin** derivatives with (3H)iodoacetate indicated that these 2 cysteines were normally disulfide-bonded. A treatment that resulted in the maximal reduction of the disulfide bond also resulted in maximal loss of cell attachment activity. These results indicate that the 76-amino acid disulfide loop at the carboxyl terminus of **invasin** is required for recognition by integrins.

L14 ANSWER 20 OF 21 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 92112957 MEDLINE
DOCUMENT NUMBER: 92112957 PubMed ID: 1730744
TITLE: The **invasin** protein of Yersinia enterocolitica: internalization of **invasin** -bearing bacteria by eukaryotic cells is associated with reorganization of the cytoskeleton.
AUTHOR: Young V B; Falkow S; Schoolnik G K
CORPORATE SOURCE: Department of Microbiology, Stanford University, California 94305.
CONTRACT NUMBER: AI26195-03 (NIAID)
GM07365 (NIGMS)
SOURCE: JOURNAL OF CELL BIOLOGY, (1992 Jan) 116 (1) 197-207.
Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202

ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 19920308
Entered Medline: 19920218

AB Yersinia enterocolitica, a facultative intracellular pathogen of mammals, readily enters (i.e., invades) cultured eukaryotic cells, a process that can be conferred by the cloned inv locus of the species. We have studied the mechanism by which the product of **inv**, a microbial outer membrane protein termed " **invasin**," mediates the internalization of bacteria by HEp-2 cells and chicken embryo fibroblasts. **Invasin**-bearing bacteria initially bound the filopodia and the leading edges of cultured cells. Multiple points of contact between the bacterial surface and the surface of the cell ensued and led to the internalization of the bacterium within an endocytic vacuole; the same multi-step process could be induced by an inert particle coated with **invasin**-containing membranes. Both adherence and internalization were blocked by an antisera directed against the beta 1 integrin cell-adherence molecule. Ultrastructural studies of **detergent**-insoluble cytoskeletons from infected cells and immunofluorescence microscopy of phalloidin-labeled cells showed alterations in the structure of the cytoskeleton during the internalization process including the accumulation of polymerized actin around entering bacteria. Bacterial entry was prevented by cytochalasin D indicating that the internalization process requires actin microfilament function. Possible linkages between beta 1 containing integrins and the cytoskeleton were examined during the internalization process through the use of protein-specific antibodies and immunofluorescence microscopy. Like actin, the actin-associated proteins filamin, talin and the beta 1 integrin subunit were also found to accumulate around entering bacteria. These findings suggest that the **invasin**-mediated internalization process is associated with cytoskeletal reorganization.

L14 ANSWER 21 OF 21 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 90182674 MEDLINE
DOCUMENT NUMBER: 90182674 PubMed ID: 2311122
TITLE: Multiple beta 1 chain integrins are receptors for **invasin**, a protein that promotes bacterial penetration into mammalian cells.
AUTHOR: Isberg R R; Leong J M
CORPORATE SOURCE: Department of Molecular Biology and Microbiology, School of Medicine, Tufts University, Boston, Massachusetts 02111.
CONTRACT NUMBER: RO1-AI23538 (NIAID)
SOURCE: CELL, (1990 Mar 9) 60 (5) 861-71.
Journal code: 0413066. ISSN: 0092-8674.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199004
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19970203
Entered Medline: 19900425

AB Mammalian cell receptors that promote entry of intracellular bacteria into nonphagocytic cells have not been identified. We show here that multiple members of the integrin superfamily of cell

adhesion receptors bind the Y. pseudotuberculosis **invasin** protein prior to bacterial penetration into mammalian cells. Affinity chromatography of crude **detergent** extracts demonstrated that integrins containing the subunit structures alpha 3 beta 1, alpha 5 beta 1, and alpha 6 beta 1 bound to immobilized **invasin**. Furthermore, phospholipid vesicles containing isolated integrin proteins were able to attach to **invasin**. Specificity for **invasin** binding to the identified integrin receptors was also demonstrated, as immunoprobing and phospholipid reconstitution studies showed that the alpha 2 beta 1 integrin, beta 2 chain integrins, and vitronectin receptor (alpha v beta 3) were not involved in cellular attachment to **invasin**.

FILE 'HCAPLUS' ENTERED AT 11:50:35 ON 10 JUL 2003
L21 0 S L5 AND DE NATUR?

FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER' ENTERED AT 11:58:00 ON 10 JUL 2003
L22 0 S L21

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER' ENTERED AT 11:48:33 ON 10 JUL 2003)
L15 236 S "PICKING W"?/AU — Author (s)
L16 196 S "OAKS E"?/AU
L17 12 S L15 AND L16
L18 26 S (L15 OR L16) AND L5
L19 34 S L17 OR L18
L20 18 DUP REM L19 (16 DUPLICATES REMOVED)

L20 ANSWER 1 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:905739 HCAPLUS
DOCUMENT NUMBER: 137:383792
TITLE: Heterologous protection induced by immunization with Invaplex vaccine
INVENTOR(S): **Oaks, Edwin V.**; Turbyfill, Kevin R.
PATENT ASSIGNEE(S): Walter Reed Army Institute of Research, USA
SOURCE: PCT Int. Appl., 71 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002094190	A2	20021128	WO 2002-US16029	20020517
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002197276	A1	20021226	US 2002-150814	20020517

PRIORITY APPLN. INFO.: US 2001-292154P P 20010518

US 2001-292493P P 20010521

AB In this application is described a compn., Invaplex, derived from a gram neg. bacteria for use in generating an immune response in a subject against one ore more heterologous species or strains of gram-neg. bacteria. The Invaplex (**invasin** complex) vaccine comprises lipopolysaccharide, IpaB, IpaC, IpaD, VirG, 72kDa protein, and 84kDa protein. The Invaplex vaccine material can be extd. from any Shigella species or enteroinvasive E. coli. The vaccine induces IgA and IgG responses in immunized animals.

L20 ANSWER 2 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:585488 BIOSIS
DOCUMENT NUMBER: PREV200200585488
TITLE: Shigella sonnei Invaplex 50 induces heterologous protection against S. flexneri 2a in mice.
AUTHOR(S): **Oaks, E. V. (1)**; Turbyfill, K. R. (1)
CORPORATE SOURCE: (1) Walter Reed Army Institute of Research, Silver Spring, MD USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 183-184. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology Salt Lake City, UT, USA May 19-23, 2002 American Society for Microbiology . ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB Protective immunity against Shigella is primarily mediated by a serotype-specific antibody response to LPS. Protein antigens, such as the **invasin** proteins that are found in all Shigella spp., also provoke antibody responses after infection. It is conceivable that a protective immune response stimulated by protein antigens would be broadly reactive against multiple Shigella spp. Recently we have used a native subcellular **invasin** complex (Invaplex) vaccine to stimulate homologous protective immunity in guinea pigs and mice. The Invaplex consists of LPS and an increased proportion of several common protein antigens, including the **invasins** IpaB and IpaC, which seems to promote a dominant antibody response to the protein antigens. To test the effectiveness of the Invaplex vaccine in producing an immune response, both reactive and protective against a heterologous strain, mice were immunized intranasally with S. sonnei Invaplex 50 or S. flexneri 2a Invaplex 24 and subsequently challenged with either the homologous or heterologous agent. Mice immunized with S. sonnei Invaplex 50 produced antibodies to S. sonnei LPS, Ipa proteins and an 84kDa protein. Mice immunized with S. flexneri 2a Invaplex 24 (which does not contain the 84 kDa protein) produced antibodies to S. flexneri 2a LPS and the Ipa proteins. Neither monovalent vaccine stimulated antibodies to the heterologous LPS. The survival rate of S. sonnei Invaplex 50 immunized mice was 100% ($p<0.001$) for S. sonnei challenge and 89% ($p<0.001$) for S. flexneri 2a challenge. S. flexneri 2a Invaplex 24 immunized mice were protected against homologous challenge ($p<0.001$) but were not significantly protected against a heterologous S. sonnei challenge ($p=0.052$). One distinguishing characteristic of mice immunized with S. sonnei Invaplex 50 and exhibiting heterologous protection is that they produced antibodies to an 84kDa protein. Other experiments have

indicated that the 84kDa protein is exposed on the Shigella surface and is found in all 4 species of Shigella and also enteroinvasive E. coli. These studies indicate that a broadly reactive Shigella vaccine may be possible with the Invaplex 50 vaccine.

L20 ANSWER 3 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:585035 BIOSIS
DOCUMENT NUMBER: PREV200200585035
TITLE: C-terminal structure/function analysis of the IpaC **invasin** from Shigella flexneri.
AUTHOR(S): Osiecki, J. C. (1); Flentie, K. (1); **Picking, W. L. (1); Picking, W. D. (1)**
CORPORATE SOURCE: (1) University of Kansas, Lawrence, KS USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 96. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology Salt Lake City, UT, USA May 19-23, 2002 American Society for Microbiology . ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Shigella flexneri is the causative agent of shigellosis, a severe form of bacillary dysentery. Shigellosis is a serious worldwide public health problem, particularly in developing nations. Pathogen-mediated invasion of colonic epithelial cells, an essential step in Shigella pathogenesis, is mediated by the invasion plasmid antigens, which are secreted via the Shigella type III secretion system. Of the invasion plasmid antigens, IpaC is responsible for subverting host cell signaling cascades to direct the uptake of S. flexneri by epithelial cells. We have previously shown that different regions of IpaC are responsible for the specific activities observed for this protein. Computer analysis of the IpaC sequence predicts that a coiled-coil domain is located near this protein's C-terminus. This coiled-coil structure is believed to be involved in oligomerization of IpaC, an activity that may be critical for its effector function. Linker-scanning mutagenesis was used to introduce specific mutations in the C-terminal portion of IpaC (residues 309 to 341) to determine the location and orientation of critical residues involved in oligomerization and effector function. Additional site-specific mutations were then introduced to determine the importance of the immediate C-terminus of IpaC (amino acids 342 to 363). From the acquired data, the immediate C-terminal 20 amino acids of IpaC are essential for IpaC effector function, but have no role in IpaC-directed contact-mediated hemolysis activity. In contrast, amino acids 309 to 341 are required for both of these activities. Moreover, inactivating mutations within this region occur in a periodic manner, which is consistent with the presence of a coiled-coil structure. The data described here shown that IpaC's invasion and hemolysis functions are distinct and that a predicted coiled-coil structure is required for both of these activities.

L20 ANSWER 4 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:339403 BIOSIS
DOCUMENT NUMBER: PREV200100339403
TITLE: Invaplex from gram negative bacteria, method of purification and methods of use.

AUTHOR(S): **Oaks, Edwin V. (1)**; Turbyfill, Kevin Ross
CORPORATE SOURCE: (1) Gambrills, MD USA
ASSIGNEE: The United States of America as represented by the Secretary of the Army
PATENT INFORMATION: US 6245892 June 12, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (June 12, 2001) Vol. 1247, No. 2, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB Invaplex, a novel composition comprising **invasin** proteins and LPS from gram-negative bacteria is described as well as methods of using the novel composition as an adjuvant and a diagnostic tool.

L20 ANSWER 5 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:201502 BIOSIS
DOCUMENT NUMBER: PREV200200201502
TITLE: Protective immunity against Shigella flexneri 2a and S. sonnei using a bivalent Shigella **invasin** complex (Invaplex) vaccine.
AUTHOR(S): **Oaks, E. V. (1)**; Turbyfill, K. R. (1)
CORPORATE SOURCE: (1) Walter Reed Army Institute of Research, Silver Spring, MD USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 311. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Protective immunity against Shigella is primarily effective against the homologous serotype. For this reason multivalent Shigella vaccines must contain LPS antigens representative of each Shigella species. Worldwide, over 160 million cases of bacillary dysentery occur annually, with the two most prevalent species being S. flexneri and S. sonnei. Recently, we described a subcellular vaccine isolated from virulent Shigella, consisting of proteins (including the **invasins** IpaB and IpaC) and LPS. This **invasin** complex (Invaplex) vaccine for S. flexneri 2a protected guinea pigs or mice from homologous challenge. Using Invaplex isolated from S. flexneri 2a and S. sonnei, a bivalent vaccine has been constructed and used to intranasally immunize mice to determine the effectiveness against challenge with either strain. Mice immunized with the bivalent S. flexneri 2a/S. sonnei Invaplex vaccine produced serum IgA and IgG serum antibodies to S. flexneri LPS, S. sonnei LPS and the water extract antigens (**invasins**) as determined by ELISAs. The immune responses in mice immunized with the bivalent vaccine were similar to responses in animals immunized with the monovalent Invaplex vaccines. Mice immunized with the bivalent vaccine were protected from a lethal lung challenge of either S. flexneri 2a (87% survivors, $p<.001$) or S. sonnei (100% survivors, $p<0.001$). Mice immunized with monovalent vaccines were protected against the homologous agent at comparable levels (S. flexneri 2a, 93%, $p<0.001$; S. sonnei, 100%, $p<0.001$). After challenge, survivors

demonstrated significant boosts in antibody titers to LPS and water extract antigens. These studies indicate that the Invaplex vaccine will be readily adaptable to a multivalent vaccine approach for shigellosis.

L20 ANSWER 6 OF 18 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2000:277999 HCAPLUS
DOCUMENT NUMBER: 132:307246
TITLE: Method for the production of purified **invasin** protein and use thereof
INVENTOR(S): **Picking, William D.; Picking, Wendy D.; Oaks, Edwin V.**
PATENT ASSIGNEE(S): St. Louis University, USA
SOURCE: PCT Int. Appl., 78 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000023462	A1	20000427	WO 1999-US24931	19991021
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1131338	A1	20010912	EP 1999-970664	19991021
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: US 1998-105085P P 19981021
US 1999-136754P P 19990601
WO 1999-US24931 W 19991021

AB A method for prodn. of highly purified **invasin** proteins is disclosed. The **invasin** proteins are recombinant IpaC or SipC derived from Shigella spp., Salmonella spp., and enteroinvasive Escherichia coli. Also disclosed are vaccine and adjuvant compns. comprising highly purified **invasin** proteins and the use of highly purified adjuvant proteins to induce an immune response and for delivery of therapeutic and diagnostic agents or drugs.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 7 OF 18 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 2000:227459 HCAPLUS
DOCUMENT NUMBER: 132:255947
TITLE: Invaplex from gram negative bacteria, method of purification and methods of use
INVENTOR(S): **Oaks, Edwin V.**; Turbyfill, Kevin Ross
PATENT ASSIGNEE(S): Walter Reed Army Institute of Research, USA
SOURCE: PCT Int. Appl., 72 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000018354	A2	20000406	WO 1999-US22771	19990929
WO 2000018354	A3	20010104		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000010984	A1	20000417	AU 2000-10984	19990929
US 6245892	B1	20010612	US 1999-408011	19990929
US 6277379	B1	20010821	US 1999-407330	19990929
US 2001009957	A1	20010726	US 2001-772878	20010131

PRIORITY APPLN. INFO.:

US 1998-102397P	P	19980930
US 1998-102398P	P	19980930
US 1999-136190P	P	19990527
US 1999-408011	A3	19990929
WO 1999-US22771	W	19990929

AB Invaplex, a novel compn. comprising **invasin** proteins and lipopolysaccharides (LPS) from gram-neg. bacteria is described as well as methods of using the novel compn. as an adjuvant and a diagnostic tool. Example bacteria are Shigella and Escherichia. Examples are given for isolation of Invaplex, immunogenicity and safety, , and adjuvanticity.

L20 ANSWER 8 OF 18 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:227460 HCAPLUS
DOCUMENT NUMBER: 132:264091
TITLE: Use of purified Invaplex from gram negative bacteria as a vaccine
INVENTOR(S): **Oaks, Edwin V.**; Turbyfill, Kevin Ross; Hartman, Antoinette Berrong
PATENT ASSIGNEE(S): Walter Reed Army Institute of Research, USA
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000018355	A2	20000406	WO 1999-US22772	19990928
WO 2000018355	A3	20001123		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD,				

RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000011999	A1	20000417	AU 2000-11999	19990928
EP 1198245	A2	20020424	EP 1999-969665	19990928
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
US 6245892	B1	20010612	US 1999-408011	19990929
US 6277379	B1	20010821	US 1999-407330	19990929
US 2001009957	A1	20010726	US 2001-772878	20010131

PRIORITY APPLN. INFO.:	US 1998-102397P	P 19980930
	US 1998-102398P	P 19980930
	US 1999-136190P	P 19990527
	WO 1999-US22772	W 19990928
	US 1999-408011	A3 19990929

AB A novel compn. comprising Invaplex from gram-neg. bacteria is described and is effective as a vaccine against gram-neg. bacterial infection.

L20 ANSWER 9 OF 18 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3

ACCESSION NUMBER:	2001:374740 HCAPLUS
DOCUMENT NUMBER:	135:151269
TITLE:	Isolation and characterization of a Shigella flexneri **invasin** complex subunit vaccine
AUTHOR(S):	Turbyfill, K. Ross; Hartman, Antoinette B.; **Oaks, Edwin V.**
CORPORATE SOURCE:	Department of Enteric Infections, Walter Reed Army Institute of Research, Silver Spring, MD, 20910-7500, USA
SOURCE:	Infection and Immunity (2000), 68(12), 6624-6632 CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER:	American Society for Microbiology
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The invasiveness and virulence of Shigella spp. are largely due to the expression of plasmid-encoded virulence factors, among which are the invasion plasmid antigens (Ipa proteins). After infection, the host immune response is directed primarily against lipopolysaccharide (LPS) and the virulence proteins (IpaB, IpaC, and IpaD). Recent observations have indicated that the Ipa proteins (IpaB, IpaC, and possibly IpaD) form a multiprotein complex capable of inducing the phagocytic event which internalizes the bacterium. We have isolated a complex of **invasins** and LPS from water-extractable antigens of virulent shigellae by ion-exchange chromatog. Western blot anal. of the complex indicates that all of the major virulence antigens of Shigella, including IpaB, IpaC, and IpaD, and LPS are components of this macromol. complex. Mice or guinea pigs immunized intranasally with purified **invasin** complex (invaplex), without any addnl. adjuvant, mounted a significant IgG and IgA antibody response against the Shigella virulence antigens and LPS. The virulence-specific response was very similar to that previously noted in primates infected with shigellae. Guinea pigs (keratoconjunctivitis model) or mice (lethal lung model) immunized intranasally on days 0, 14, and 28 and challenged 3 wk later with virulent shigellae were protected from disease.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 10 OF 18 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 2001:695616 HCAPLUS
DOCUMENT NUMBER: 135:287173
TITLE: Production of IFN-.gamma. and IL-10 to Shigella invasions by mononuclear cells from volunteers orally inoculated with a Shiga toxin-deleted Shigella dysenteriae type 1 strain. [Erratum to document cited in CA132:277904]
AUTHOR(S): Samandari, Taraz; Kotloff, Karen L.; Losonsky, Genevieve A.; **Picking, William D.**; Sansonetti, Philippe J.; Levine, Myron M.; Sztein, Marcelo B.
CORPORATE SOURCE: Center for Vaccine Development, Departments of Pediatrics and Medicine, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SOURCE: Journal of Immunology (2000), 165(8), 4756
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In Table III, the columns designating the Ab classes were incorrectly aligned; each heading was shifted one column to the left. The correct alignment is given. On page 2223, the first sentence in Results should now read: "A total of 23 volunteers ingested SC595. Two volunteers were excluded from anal. One volunteer in the 7 .times. 103 CFU dose group withdrew from the study 10 days after challenge and did not provide postvaccination specimens for CMI anal. Another volunteer in the 5 .times. 104 CFU dose group was excluded from anal. because repeated stool cultures during the study were devoid of all normal fecal flora possibly related to azithromycin ingestion for bronchitis 3 wk prior to challenge with SC595.".

L20 ANSWER 11 OF 18 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 5
ACCESSION NUMBER: 2000:138604 HCAPLUS
DOCUMENT NUMBER: 132:277904
TITLE: Production of IFN-.gamma. and IL-10 to Shigella **invasins** by mononuclear cells from volunteers orally inoculated with a Shiga toxin-deleted Shigella dysenteriae type 1 strain
AUTHOR(S): Samandari, Taraz; Kotloff, Karen L.; Losonsky, Genevieve A.; **Picking, William D.**; Sansonetti, Philippe J.; Levine, Myron M.; Sztein, Marcelo B.
CORPORATE SOURCE: Center for Vaccine Development, Departments of Pediatrics and Medicine, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SOURCE: Journal of Immunology (2000), 164(4), 2221-2232
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Volunteers were orally administered invasive, non-Shiga toxin-producing S. dysenteriae 1 to establish a challenge model to

assess vaccine efficacy. In stepwise fashion, 4 sep. groups were given 3.times.102, 7.times.103, 5.times.104, or 7.times.105 CFU. Using PBMC, proliferative responses and cytokine prodn. were measured to S. dysenteriae whole-cell prepns. and to purified recombinant invasion plasmid antigens (Ags) (Ipa) C and IpaD. Anti-LPS and anti-Ipa Abs and Ab-secreting cells were also evaluated. Preinoculation PBMC produced considerable quantities of IL-10 and IFN-.gamma., probably secreted by monocytes and NK cells, resp., of the innate immune system. Following inoculation, PBMC from 95 and 87% of volunteers exhibited an increased prodn. of IFN-.gamma. and IL-10, resp., in response to Shigella Ags. These increases included responses to IpaC and IpaD among those volunteers receiving the lowest inoculum. No IL-4 or IL-5 responses were detected. Whereas there were no Ab or Ab-secreting cell responses in volunteers receiving the lowest inoculum, other dose groups had moderate to strong anti-LPS and anti-Ipa responses. Thus, in humans, type 1 responses play an important role in mucosal and systemic immunity to S. dysenteriae 1.

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 12 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:325952 BIOSIS
DOCUMENT NUMBER: PREV199900325952
TITLE: Isolation and characterization of the Shigella **invasin** complex and use as a new subunit vaccine.
AUTHOR(S): Turbyfill, K. R. (1); **Oaks, E. V. (1)**; Hartman, A. B. (1)
CORPORATE SOURCE: (1) Walter Reed Army Institute of Research, Washington, DC USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 291. Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology . ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L20 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:325931 BIOSIS
DOCUMENT NUMBER: PREV199900325931
TITLE: Evaluation of the Shigella **invasin** complex and purified IpaC as mucosal adjuvants.
AUTHOR(S): **Oaks, E. V. (1)**; Turbyfill, K. R. (1); Picking, B.; **Picking, W.**
CORPORATE SOURCE: (1) Walter Reed Army Institute of Research, Washington, DC USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 282. Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology . ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L20 ANSWER 14 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:284901 BIOSIS
DOCUMENT NUMBER: PREV199799584104
TITLE: Serologic response to invasion plasmid antigens and lipopolysaccharide among individuals infected with Shigella sonnei.
AUTHOR(S): Strockbine, N. A. (1); Fernandez, S. V. (1); Mahon, B. (1); **Oaks, E. V.**; **Picking, W.**; Mintz, E. D. (1)
CORPORATE SOURCE: (1) CDC, Atlanta, GA USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1997) Vol. 97, No. 0, pp. 577.
Meeting Info.: 97th General Meeting of the American Society for Microbiology Miami Beach, Florida, USA May 4-8, 1997
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

L20 ANSWER 15 OF 18 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97118931 MEDLINE
DOCUMENT NUMBER: 97118931 PubMed ID: 8954886
TITLE: Cloning, expression, and affinity purification of recombinant Shigella flexneri invasion plasmid antigens IpaB and IpaC.
AUTHOR: **Picking W L**; Mertz J A; Marquart M E; **Picking W D**
CORPORATE SOURCE: Department of Biology, Saint Louis University, Missouri 63103-2010, USA.. pickinwd@sluaxa.slu.edu
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1996 Dec) 8 (4) 401-8.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
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AB Shigella flexneri and related enteropathogenic bacteria are important agents of bacillary dysentery, a potentially life-threatening illness for children in underdeveloped regions of the world. Onset of shigellosis stems from S. flexneri invasion of colonic epithelial cells, leading to localized cell death and inflammation. Invasion plasmid antigens (Ipa) B, C, and D are three secreted proteins encoded by the large virulence plasmid of S. flexneri that have been implicated as essential effectors of this cell invasion process. These proteins are expressed as part of the ipa operon and are among the major targets of the host immune response to shigellosis. Biochemical characterization of the Ipa **invasins** has been complicated by the fact they have not been purified in the quantities needed for detailed in vitro analysis. Here we describe the first cloning, expression, and extensive purification of IpaB and IpaC fusion proteins from Escherichia coli for use in dissecting of the protein biochemistry of S. flexneri

pathogenesis. A variety of approaches were used to prepare significant quantities of these proteins in their soluble forms, including the use of different host cell lines, modification of bacterial growth conditions, and the use of alternative plasmid expression vectors. Now that these Ipa proteins are available in a highly pure form, it will be possible to initiate studies on their important biological and immunological properties as well as their recruitment into high-molecular-weight protein complexes. Together with IpaD (purified as part of a previous study), these purified proteins will be useful for: (a) exploring properties of the host immune response to S. flexneri invasion, (b) elucidating the specific biochemical properties that lead to pathogen internalization, (c) analyzing the importance of specific Ipa protein complexes in host cell invasions, and (d) monitoring, or perhaps even augmenting, the efficacy of live oral vaccines in human trials.

L20 ANSWER 16 OF 18 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 7
ACCESSION NUMBER: 1996:232189 HCAPLUS
DOCUMENT NUMBER: 124:286512
TITLE: Antibody response of monkeys to invasion plasmid antigen D after infection with Shigella spp.
AUTHOR(S): **Oaks, Edwin V.; Picking, William D.; Picking, Wendy L.**
CORPORATE SOURCE: Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC, 20307, USA
SOURCE: Clinical and Diagnostic Laboratory Immunology (1996), 3(2), 242-5
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The antigen prepn. most often used for detg. the levels of antibodies to virulence-assocd. proteins of Shigella spp. consists of a mixt. of proteins (including IpaB, IpaC, IpaD, and VirG*) extd. from virulent shigellae with water (water ext.). To overcome the lack of specificity for individual antigens in the water-ext. ELISA, the ipaD gene from S. flexneri has been cloned, expressed to a high level, and purified for use in a new ELISA for the detn. of the levels of antibody against IpaD in monkeys and humans challenged with shigellae. The IpaD ELISA for serum Igs G and A correlated well with the water-ext. ELISA in that monkeys infected with S. flexneri or S. sonnei responded with high serum antibody titers in both assays. The IpaD assay required less antigen per well, had much lower background levels, and did not require correction with antigens from an avirulent organism. In conjunction with the water-ext. ELISA, it was possible to identify infected animals that did not respond to IpaD but did produce antibodies that reacted in the water-ext. ELISA. This indicates that even though IpaB, IpaC, and IpaD are essential for the invasiveness phenotype, the infected host does not always produce antibodies against all components of the invasiveness app.

L20 ANSWER 17 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:290397 BIOSIS
DOCUMENT NUMBER: PREV199598304697
TITLE: Serum Antibody Response Against Purified, Recombinant

	Invasion Plasmid Antigen (Ipa) D from Shigella flexneri.
AUTHOR(S):	**Oaks, E. (1); Picking, W. L.; Picking, W. D.**
CORPORATE SOURCE:	(1) Walter Reed Army Inst. Res., Washington, DC USA
SOURCE:	Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 188. Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995 ISSN: 1060-2011.
DOCUMENT TYPE:	Conference
LANGUAGE:	English

L20 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER:	1994:330585 BIOSIS
DOCUMENT NUMBER:	PREV199497343585
TITLE:	Structural features of Shigella flexneri invasion plasmid antigen D.
AUTHOR(S):	Marquart, M. (1); **Picking, W. L.; Oaks, E. V.**; Agarwal, R.; **Picking, W. D.**
CORPORATE SOURCE:	(1) Dep. Biol., Saint Louis, Univ., St. Louis, MO USA
SOURCE:	Abstracts of the General Meeting of the American Society for Microbiology, (1994) Vol. 94, No. 0, pp. 92. Meeting Info.: 94th General Meeting of the American Society for Microbiology Las Vegas, Nevada, USA May 23-27, 1994 ISSN: 1060-2011.
DOCUMENT TYPE:	Conference
LANGUAGE:	English

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